

09/367496

WO 98/37192

PCT/FR98/00328

Use of ULIP proteins in the diagnosis and therapy of
cancers and paraneoplastic neurological syndromes

Sute BT The invention relates to the use of proteins designated ULIP/POP in the diagnosis and therapy of
5 cancers and paraneoplastic neurological syndromes.

Paraneoplastic neurological syndromes (PNS) occur in the instance of a cancer, often before its discovery, and are not connected either to the tumour proliferation itself (direct invasion, metastases) or
10 to the therapy. Their frequency is globally estimated at approximately 1% of cancers. Several clinical pictures have been individualized for a long time (encephalomyelitis, Denny-Brown's sensitive neuropathy, cerebellar atrophy, limbic encephalitis,
15 opsoclonus,...) corresponding in fact to the either elective or preferential attack of certain groups of neurons. The frequency of inflammatory cells in the neighbourhood of the lesions for numerous years brought to mind the possibility of an auto-immune or viral
20 process. The more recent demonstration of auto-antibodies in the serum and the cerebrospinal fluid (CSF) of patients suffering from PNS, specific to the type of tumour and the type of neurons which degenerate, has revived the hypothesis of participation
25 of auto-immunity in the genesis of this pathology (Graus et al., 1985; Greenlee et al., 1983).

Apart from the presence of a high titre of these antibodies in the blood and the CSF of patients, there are several arguments suggesting that PNS depend
30 on auto-immune mechanisms. Thus the antigens recognized in the central nervous system are also present in the tumours of patients (Anderson et al., 1987). At the level of the tumour tissue, antibodies specifically directed against these antigens as well as B and T
35 lymphocytes are found (Hetzl et al., 1990).

These data suggest that the auto-immune process could be triggered by the expression of tumour antigens. A cross-immunity process could provoke the lesions of the central nervous system. Other arguments

- 2 -

additionally indicate that the cerebral lesions result from the auto-immune response. Thus, in the brain of the patients, the titre of specific antibodies is higher than that of the serum and the CSF (Dalmau et al., 1991). In addition, in the case of encephalomyelitis associated with anti-Hu antibodies, there is an intense lymphocytic reaction, made up of B and T cells, situated in proximity to neurons in the process of destruction (Dalmau et al., 1991; Graus et al., 1990).

Several types of auto-antibodies allowing precise syndromic groupings as a function of immunological, neurological and carcinogenic criteria have been described.

Thus, anti-Yo antibodies are found in the serum and the CSF of women having paraneoplastic cerebellar atrophy and a gynaecological cancer (ovary, breast or uterus) (Greenlee et al., 1983; Jaekle et al., 1985).

These antibodies recognize two cytoplasmic proteins of 34 and 62 kDa specific to Purkinje cells of the cerebellum.

The anti-Ri antibodies are found in the serum and the CSF of patients (principally of women) having opso-myoclonus, cerebellar syndrome and breast cancer. These antibodies recognize two proteins of 50 and 80 kDa specific to neurons of the central nervous system (Luque et al., 1991).

Anti-Hu antibodies are most frequently found in the course of PNS. They are found in the serum and the CSF of patients having Denny-Brown's syndrome or encephalomyeloneuritis and small-cell lung cancer (Graus et al., 1985; Dalmau et al., 1992). These auto-antibodies recognize several proteins of 37 to 45 kDa expressed specifically by all the neurons of the nervous system.

Another type of auto-antibody has recently been identified in patients having PNS: anti-CV2 antibodies (Antoine et al., 1993; Honnorat et al., 1996). The latter are atypical, in the sense that the antigenic

- 3 -

target recognized in adulthood is essentially non-neuronal, although the *post-mortem* analysis of the brain of four patients allows neuronal loss, gliosis and an inflammatory process characteristic of PNS to be objectivized.

The originality of the discovery of these auto-antibodies resides, on the one hand, in their demonstration. The latter escaped all the usual investigations which consisted in revealing the antigens recognized by immunohistochemistry on *post-mortem* brain. The antigen recognized is indeed soluble and disappears from *post-mortem* brain under the majority of fixation conditions. Only fixation of human *post-mortem* tissue by immersion in paraformaldehyde or *in situ* by perfusion of paraformaldehyde in animals has allowed the presence of these antibodies in the CSF or the serum of patients suffering from PNS to be revealed (Antoine et al., 1993; Honnorat et al., 1996).

The anti-CV2 auto-antibodies present in the sera of patients suffering from paraneoplastic neurological syndrome (PNS) have been defined by their capacity to recognize, by indirect immunohistochemistry, a cytoplasmic antigen expressed specifically, in adult rat brain, by a subpopulation of oligodendrocytes of the brain stem, the medulla and the cerebellum.

The originality of these auto-antibodies resides, on the other hand, in their diagnostic interest. Their presence in the serum or the CSF of patients is of diagnostic value because it allows the paraneoplastic origin of a neurological syndrome to be specified. The discovery of these antibodies, when it precedes that of cancer, directs the search to that and allows its discovery. Such was the case for six patients out of 19 having anti-CV2 antibodies. The clinical disorders were different according to the patients, certain of them having a picture of limbic encephalitis, others encephalomyeloneuritis and others Lambert-Eaton syndrome. Nevertheless, in more than 60%

- 4 -

of the cases, the cerebellar syndrome was predominant. The most frequently associated tumour was small-cell lung cancer (60% of the cases).

5 Experiments on the brains of newborn rats showed that these anti-CV2 antibodies reacted with a protein of 66 kDa (Honnorat et al., 1996).

10 In the adult brain, this antigen is situated in a subpopulation of oligodendrocytes or in cells which retain differentiation capacities in the adult brain (olfactory bulb, dentate gyrus). The recognized antigen could play a role in neuronal survival, via Neuron/Oligodendrocyte interactions, as the loss of neurons observed in the post-mortem brain of patients suffering from PNS suggests.

15 Its very limited expression in adulthood contrasts with a very strong and transitory expression in the central and peripheral nervous system in development, suggesting the probable role of this antigen in the development of the nervous system.

20 The Applicant has characterized the target antigen of anti-CV2 antibodies, which corresponds to a protein designated below by "POP-66" for "paraneoplastic oligodendrocyte protein 66 kDa".

25 Surprisingly, it has been discovered that the POP-66 protein belongs to the so-called ULIP family of proteins (for Unc-33-like phosphoprotein), involved in the control of neuronal development and axonal transport (T. Byk et al., 1996), and also studied in the form of CRMP proteins (Goshima et al., 1995, Wang et al., 1996), TOAD-64 (Minturn et al., 1995) and DRPs (Hamajima et al., 1996). More precisely, POP-66 has been identified as in fact being the human form of ULIP-4.

30 All of the data described below emphasize the complexity of this family of proteins, the existence of a very wide expression spectrum of members of this family in the brain in the course of ontogenesis, but a very limited spectrum in adults, as well as the

- 5 -

ms.
B2

specificity of the anti-CV2 antibodies for a member of this ULIP protein family, which is in fact POP-66.

Thus, the Applicant has shown that the protein recognized by the anti-CV2 antibodies of patients suffering from PNS is POP-66/ULIP-4 and has established the involvement of the ULIP proteins in paraneoplastic neurological syndromes and associated cancers. In addition to their role in cancers associated with PNS, the Applicant has likewise discovered that the proteins of the ULIP family could play a role in any other form of cancer not associated with PNS. More particularly, the ULIP proteins could especially be involved in cancers of tissues having a common embryonic origin with the central nervous system.

The present invention therefore relates to a purified ULIP polypeptide, derivative or polypeptide fragment of the said purified polypeptide, comprising an amino acid sequence selected from SEQ ID No. 2, No. 4, No. 6 and No. 8.

Preferentially, the present invention relates to a purified polypeptide, derivative, or biologically active polypeptide fragment of the said purified polypeptide, comprising the amino acid sequence SEQ ID No. 8, the said polypeptide being designated by "POP-66/ULIP-4".

A fragment of the polypeptide of sequence SEQ ID No. 8 of interest is, in particular, the antigenic fragment PARASCPGKIS (amino acids No. 517 to No. 527).

In the description of the invention, the following definitions are used:

- derivative: any variant polypeptide of the polypeptide of sequence SEQ No. 2, No. 4, No. 6 or No. 8 or any other molecule resulting from a modification of genetic and/or chemical nature of the sequence SEQ ID No. 2, No. 4, No. 6 or No. 8, that is to say obtained by mutation, deletion, addition, substitution and/or chemical modification of a single or of a limited number of amino acids, as well as any

- 6 -

isoform sequence, that is to say a sequence identical to the sequence SEQ ID No. 2, No. 4, No. 6 or No. 8, to one of its fragments or modified sequences, containing one or more amino acids in the D enantiomer form, the
5 said modified or isoform variant sequences having conserved at least one of the properties making them biologically active.

- biologically active: having properties of induction and/or control of neuronal development and/or
10 antigenic properties.

The invention likewise relates to an isolated nucleic acid sequence selected from SEQ ID No. 1, No. 3, No. 5 and No. 7 or a sequence derived from the sequences SEQ ID No. 1, No. 3, No. 5 and No. 7 on
15 account of the degeneracy of the genetic code, or on account of mutation, of deletion or of insertion of at least one nucleotide, the said derived sequences having a biological activity virtually identical to that of the peptide encoded by the sequences SEQ ID No. 1,
20 No. 3, No. 5 and No. 7.

The various nucleotide sequences of the invention can be of artificial or non-artificial origin. They can be DNA or RNA sequences, obtained by screening of banks of sequences by means of probes
25 elaborated on the basis of sequences selected from SEQ ID No. 2, No. 4, No. 6 and No. 8. Such banks can be prepared by conventional techniques of molecular biology known to the person skilled in the art.

The nucleotide sequences according to the
30 invention can likewise be prepared by chemical synthesis, or alternatively by mixed methods including the chemical or enzymatic modification of sequences obtained by screening of banks.

These nucleotide sequences allow the production
35 of nucleotide probes capable of hybridizing strongly and specifically with a nucleic acid sequence of a genomic DNA or of a messenger RNA coding for a peptide according to the invention or a biologically active fragment of this. The appropriate hybridization

- 7 -

conditions correspond to the conditions of temperature and of ionic strength usually used by the person skilled in the art (Sambrook et al., 1989), preferably to temperature conditions of between (T_m minus 5°C) and 5 T_m minus 30°C) and more preferably to temperature conditions of between (T_m minus 5°C) and (T_m minus 10°C) (great stringency), T_m being the theoretical melting point, defined as being the temperature at which 50% of the paired strands separate. Such probes are likewise 10 part of the invention. They can be used as a diagnostic tool *in vitro* for the detection, by hybridization experiments, of specific transcripts of polypeptides of the invention in biological samples or for the demonstration of aberrant syntheses or genetic 15 anomalies resulting from polymorphism, mutations or bad splicing.

The probes of the invention contain at least 10 nucleotides, and at most contain the whole of a nucleotide sequence selected from SEQ ID No. 1, No. 3, 20 No. 5 and No. 7 or of their complementary strand.

The *in vitro* diagnostic methods in which these nucleotide probes are employed for the detection of aberrant syntheses or genetic anomalies, such as the loss of heterozygosity and genetic rearrangement, at 25 the level of nucleic sequences coding for a ULIP polypeptide according to the invention or a biologically active fragment are included in the present invention. Such a method type comprises:

- the contacting of a nucleotide probe of the 30 invention with a biological sample under conditions allowing the formation of a hybridization complex between the said probe and the abovementioned nucleotide sequence, optimally after a previous amplification step of the abovementioned nucleotide 35 sequence;

- the detection of the hybridization complex optimally formed;

- 8 -

- optimally the sequencing of the nucleotide sequence forming the hybridization complex with the probe of the invention.

The cDNA probes of the invention can additionally be advantageously used for the detection of chromosomal anomalies.

The nucleotide sequences according to the invention are likewise useful for the production and use of sense and/or antisense oligonucleotide primers for sequencing reactions or specific amplification reactions according to the so-called PCR technique (polymerization chain reaction) or any other variant of this.

The nucleotide sequences according to the invention additionally have uses in the therapeutic field, for the production of antisense sequences capable of hybridizing specifically with a nucleic acid sequence, including a messenger RNA, which can be used in gene therapy. The invention thus relates to antisense sequences capable of inhibiting, at least partially, the production of a polypeptide according to the invention, such as defined above.

They are more particularly useful in the treatment of disorders of the central and peripheral nervous system and of vision, especially in the treatment of paraneoplastic neurological syndromes, as well as in anti-cancer treatment, especially of tumours associated with paraneoplastic neurological syndromes.

The nucleotide sequences according to the invention can additionally be used for the production of recombinant ULIP proteins according to the invention.

These proteins can be produced from nucleotide sequences defined above, according to techniques of production of recombinant products known to the person skilled in the art. In this case, the nucleotide sequence used is placed under the control of signals allowing its expression in a cell host.

- 9 -

An efficacious system of production of a recombinant protein necessitates having a vector, for example of plasmid or viral origin, and a compatible host cell.

5 The cell host can be selected from prokaryotic systems, such as bacteria, or eukaryotic systems, such as, for example, yeasts, insect cells, CHO (Chinese hamster ovary) cells or any other system advantageously available. A preferred cell host for the expression of
10 proteins of the invention is formed by the bacterium *E. coli*.

The vector must contain a promoter, translation initiation and termination signals, as well as the appropriate regions of transcription regulation. It
15 must be able to be maintained stably in the cell and can possibly possess special signals specifying the secretion of the translated protein.

These different control signals are selected as a function of the cell host used. To this end, the
20 nucleotide sequences according to the invention can be inserted in autonomous replication vectors within the selected host, or integrative vectors of the selected host. Such vectors will be prepared according to methods currently used by the person skilled in the
25 art, and the resulting clones can be introduced into an appropriate host by standard methods, such as, for example, electroporation.

The invention is additionally directed at the host cells transfected by these above vectors. These
30 cells can be obtained by the introduction into host cells of a nucleotide sequence inserted into a vector such as defined above, then the culturing of the said cells under conditions allowing the replication and/or expression of the transfected nucleotide sequence.

35 These cells can be used in a method of production of a recombinant polypeptide according to the invention or any fragment or biologically active derivative of this.

- 10 -

The method of production of a polypeptide of the invention in recombinant form is itself included in the present invention, and is characterized in that the transfected cells are cultured under conditions
5 allowing the expression of a recombinant polypeptide according to the invention or of any fragment or biologically active derivative of this, and in that the said recombinant polypeptide is recovered.

The purification processes used are known to
10 the person skilled in the art. The recombinant polypeptide can be purified from lysates and cell extracts, from the supernatant of the culture medium, by methods used separately or in combination, such as fractionation, chromatographic methods, immunoaffinity
15 techniques with the aid of specific mono- or polyclonal antibodies, etc:

One variant consists in producing a recombinant polypeptide fused to a "carrier" protein (chimeric protein). The advantage of this system is that it
20 allows a stabilization and a decrease in the proteolysis of the recombinant product, an increase in the solubility in the course of the *in vitro* renaturation and/or a simplification of the purification when the fusion component has an affinity
25 for a specific ligand.

The exploitation of ULIP proteins, and in particular POP-66/ULIP-4, as well as antibodies directed against these proteins, is promising in various fields.

30 Thus, the detection of the anti-CV2 auto-antibody by immunofluorescence on fixed animal brain is currently used as a diagnostic test.

The production of POP-66/ULIP-4 recombinant protein according to the invention allows the
35 production of a rapid and reliable test (of Elisa or Western Blot type) for detecting anti-CV2 antibodies.

Such tests already exist for anti-Hu, anti-Yo and anti-Ri antibodies. The test for detecting anti-CV2 in the serum of patients could be prescribed in the

- 11 -

case of suspicion of paraneoplastic neurological syndrome and consequently could include anti-CV2 antibodies at the same titre as the other antibodies identified in the PNS such as mentioned above.

5 The invention is therefore likewise directed at a method for the diagnosis of paraneoplastic neurological syndromes and/or for the early diagnosis of the formation of tumours of cancerous origin, characterized in that auto-antibodies directed against
10 a POP-66/ULIP-4 protein are demonstrated in a blood sample taken from an individual by

- the contacting of a blood sample taken from an individual with a purified polypeptide (POP-66), derivative or biologically active polypeptide fragment
15 of POP-66/ULIP-4 optionally attached to a support under conditions allowing the formation of specific immunological complexes between the said polypeptide and the auto-antibodies optionally present in the serum sample, and

20 - the detection of the specific immunological complexes optionally formed.

The invention likewise relates to a kit for the diagnosis of paraneoplastic neurological syndromes and for the early diagnosis of the formation of tumours
25 from a biological sample, comprising:

- at least one purified POP-66/ULIP-4 polypeptide, derivative or biologically active polypeptide fragment of POP-66/ULIP-4, optionally attached to a support,

30 - means of visualization of the formation of specific antigen/antibody complexes between an anti-POP-66 auto-antibody and the said purified POP-66 polypeptide, derivative or polypeptide fragment and/or means of quantification of these complexes.

35 The invention likewise relates to mono- or polyclonal antibodies or their fragments, chimeric or immunoconjugated antibodies obtained from a purified ULIP polypeptide comprising an amino acid sequence selected from SEQ ID No. 2, No. 4, No. 6 and No. 8,

- 12 -

derivative or biologically active polypeptide fragment of ULIP and their use for the purification or the detection of a ULIP protein in a biological sample.

Polyclonal antibodies can be obtained from the serum of an animal immunized against the protein, produced, for example, by genetic recombination according to the method described above, according to the usual working methods.

The monoclonal antibodies can be obtained according to the conventional method of hybridoma culture described by Köhler and Milstein.

The antibodies can be chimeric antibodies, humanized antibodies, Fab and F(ab')₂ fragments. They can likewise be present in the form of immunoconjugates or labelled antibodies.

The invention likewise relates to the use of antibodies directed against a protein of the ULIP family for the demonstration of a ULIP protein in neoplasms, and paraneoplastic neurological syndromes for diagnostic purposes.

Preferentially, the invention relates to the use of monoclonal antibodies obtained from polyclonal anti-CV2 serum of patients by immortalization of lymphocytes, according to the usual techniques known to the person skilled in the art.

Thus, the antibodies directed against a protein of the ULIP family are useful for detecting abnormal expression of ULIP protein in patients having neurological syndromes, in whom cancer has not been diagnosed by the conventional methods. This abnormal expression of ULIP protein will be able to be correlated with the existence of a cancer which had not been spotted. Thus, the antibodies directed against a ULIP protein, especially against POP-66/ULIP-4, are useful for the early diagnosis of cancer.

The invention likewise relates to a method of determination of an allelic variability, a mutation, a deletion, an insertion, a loss of heterozygosity or a genetic anomaly of the POP-66/ULIP-4 gene, situated on

- 13 -

chromosome 10 in the 26q region and which can be involved in pathologies, characterized in that it employs at least one nucleotide sequence SEQ ID No. 7. Amongst the methods of determination of an allelic variability, a mutation, a deletion, an insertion, a loss of heterozygosity or a genetic anomaly of the POP-66/ULIP-4 gene, a method comprising at least one PCR amplification step of the nucleic sequence of POP-66/ULIP-4 capable of having a polymorphism, a mutation, a deletion or an insertion with the aid of pairs of primers of nucleotide sequences, a step in the course of which amplified products are treated with the aid of appropriate restriction enzymes and a step in the course of which at least one of the products of the enzymatic reaction is detected or determined is preferred.

Advantageously, it is possible to search for the mutations associated with the said chromosome 10 in relation to cancer, especially peripheral cancerous tumours and primitive cerebral tumours of glial origin, for example.

The invention likewise relates to a pharmaceutical composition comprising at least one purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

The invention preferentially comprises pharmaceutical compositions comprising as active principle a purified POP-66 polypeptide, derivative or polypeptide fragment of POP-66, preferentially in soluble form, combined with a pharmaceutically acceptable vehicle.

Such compositions offer a new approach to treating disorders of the central and peripheral

- 14 -

nervous system and of vision, and especially paraneoplastic neurological syndromes. In addition, they are useful for treating neurological disorders connected with a neuronal loss and/or an underexpression of ULIP proteins in the nervous system.

Thus, POP-66/ULIP-4 is also of interest in neurodegenerative pathologies such as multisystemic atrophies which are conditions similar to those of PNS and for which an anomaly of an oligodendrocytic subpopulation has been detected (Papp et al., 1992).

The compositions according to the invention are additionally useful in anticancer therapy.

The antibodies directed against one or more ULIP proteins can be combined with antineoplastic agents, thus allowing the targeting of medicaments towards the tumour cells.

They can additionally be combined with a hydrophilic chemical group chosen in such a way so as to cross or not to cross the blood-brain barrier, according to the type of tumour.

The ULIP proteins and in particular POP-66 as well as the nucleotide sequences coding for the said proteins and the antisense sequences or oligonucleotides can be useful in the therapy of any type of cancer in which a gene coding for a ULIP protein is involved. Amongst examples of cancers, it is possible to mention peripheral tumours, such as small-cell lung cancer, thymoma, cancer of the breast and of the ovary, as well as cerebral tumours, preferably primitive cerebral tumours of glial origin. The expression of POP-66 in the non-proliferative cells of normal brain, its absence in normal tissues such as lung or thymus, for example, its differential reexpression during tumorigenesis of these tissues and the modulation of its expression in a tumour line in the course of differentiation suggest in this respect that POP-66 could be a tumour suppressor gene.

Preferentially, the pharmaceutical compositions according to the invention can be administered by the

- 15 -

systemic route, preferably by the intravenous route, by the intramuscular route, intradermally or by the oral route.

5 Their modes of administration, dosages and optimal pharmaceutical forms can be determined according to the criteria generally taken into account in the establishment of a therapeutic treatment adapted to a patient, such as, for example, the age or the body weight of the patient, the seriousness of his/her
10 general condition, the tolerance to the treatment and the secondary effects noted, etc.

The invention likewise comprises the use of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a
15 nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a
20 pharmaceutically acceptable vehicle, for the production of a medicament intended for treating neurodegenerative illnesses and neoplasms.

The invention finally relates to a method of treatment of neurodegenerative illnesses and neoplasms,
25 comprising the administration to a subject requiring such a treatment of a therapeutically efficacious quantity of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence
30 fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

35 The examples and the figures whose legends are presented below are given by way of illustration.

- 16 -

LEGEND TO THE FIGURES

ms
B3

- Figure 1 represents a two-dimensional electrophoresis profile obtained from brain protein extracts of newborn rats enriched in POP-66.

A: silver staining of all of the proteins.

B: immunoblot with the anti-CV2 serum of patients.

The arrows indicate the spots corresponding to POP-66, revealed with anti-CV2 antibodies.

- Figure 2 represents a two-dimensional electrophoresis profile obtained from protein extracts of brains of newborn rats.

Immunoblot with A- antipeptide antibody 3 and B- anti-CV2 antibody.

- Figure 3 represents a one-dimensional electrophoresis obtained from protein extracts of brains of newborn rats.

Immunoblot with a: preimmune serum for peptide 3

Immunoblot with b: anti-peptide serum 3

Immunoblot with c: anti-peptide serum 4

Immunoblot with d: preimmune serum for peptide 4.

- Figure 4 represents an immunohistochemical labelling of sections of brains of adult rats with

A: anti-CV2 serum of a patient suffering from PNS

B: rabbit serum with anti-peptide 3 antibodies

C: rabbit serum with anti-peptide 4 antibodies.

- Figure 5 represents a histological labelling of sections of young rat cerebellum 8 days post-natally.

A: Staining with toluidine blue; ge = external granular layer; m = molecular layer (x400).

B: Immunolabelling after incorporation of BrdU (bromodeoxyuridine). The cells which have incorporated BrdU are virtually all situated in the most external zone of the external granular layer (ge). Some positive cells are situated in the internal granular layer (x400).

- 17 -

C: Indirect immunoperoxidase with a patient serum containing an anti-CV2 antibody (x400). The immunoreactivity is concentrated in the internal part of the external granular layer (future molecular layer (m)). Some cells are immunoreactive in the internal granular layer. The Purkinje cells (p) are negative as well as the cells of the external part of the external granular layer (ge).

D: Indirect immunoperoxidase with a patient serum containing an anti-CV2 antibody (x1000). Above all, the immunolabelling is concentrated in the internal part of the external granular layer (future molecular layer (m)). A reactive cell is noted in the internal granular layer (gi) (arrow).

- Figure 6 represents immunohistochemical labelling of sections of post-mortem hippocampus (HPS staining).

A: brain of control patient,

B: brain of patient having limbic encephalitis, and circulating anti-CV2 antibody. It is possible to note the disappearance of the granular cells.

- Figure 7 represents a two-dimensional electrophoresis profile with the control ULIP-2 protein (A) and the ULIP-4 protein (B).

Figure 7C represents the migration profile model of the proteins ULIP-1, 2, 3 and 4 as a reference.

The proteins are revealed:

a) by autoradiography to locate the proteins translated *in vitro* (translation);

b) by immunoblotting with the anti-CV2 serum.

- Figure 8 represents a migration profile of the mRNA of C-22/ULIP-3 (8A) and TOAD-64/ULIP-2 (8B) amplified by RT-PCR expressed in different cell types:

lanes 1-3:	small-cell lung tumour
lane 2:	small-cell lung tumour with anti-CV2 serum
lane 4:	control cDNA.

- 18 -

lane 5: medulloblastoma treated by
HTLV1 infection

lanes 6-7: medulloblastoma

lane 8: C6 line of glial cells in mice

5 lane 9: control

lane 10: nothing

lane 11: kb scale.

10 The black arrows correspond to POP-66; the
white arrows correspond to the molecular weight
standard.

- Figure 9 represents the nucleotide sequence
of ULIP-2 in mice (SEQ ID No. 1), as well as the
inferred amino acid sequence (SEQ ID No. 2).

15 - Figure 10 represents the nucleotide sequence
of ULIP-3 in mice (SEQ ID No. 3), as well as the
inferred amino acid sequence (SEQ ID No. 4).

- Figure 11 represents the nucleotide sequence
of ULIP-4 in mice (SEQ ID No. 5), as well as the
inferred amino acid sequence (SEQ ID No. 6).

20 - Figure 12 represents the nucleotide sequence
of ULIP-4 in man (SEQ ID No. 7), as well as the
inferred amino acid sequence (SEQ ID No. 8).

25 An erroneous stop codon in the human ULIP-4
sequence (asterisk) arises from a fault of the reverse
transcriptase in the production of the bank. By
comparing with ULIP-4 of rats and of mice, it is almost
certain that the TAG sequence coding for a stop is in
fact an AAG codon, coding for a lysine as in rats and
mice. In addition, the region around this amino acid is
30 entirely conserved in the three species.

Suk
B4
The amino acid sequence has been completed in
SEQ ID No. 8 by 15 C-terminal amino acids (No. 554 to
No. 568). This C-terminal region which is missing in
Figure 12 is very well conserved between rat and mice
35 ULIP-4 as well as between the different ULIPs.

Ms. B⁵

- 19 -

EXAMPLE 1:Purification of POP-66 and sequencing

5 The purification of POP-66 is carried out according to the material and the methods described in the article of Honnorat et al., 1996, incorporated by reference, starting from serum of patients suffering from PNS.

10 To identify the protein POP-66, a purification strategy was chosen which allows a partial sequencing to be obtained. The screening of an expression bank of brain cDNA or the immunoaffinity purification of the protein were excluded because of the limited quantities of sera linked to the death of the patients. It was
15 possible to develop a method of biochemical purification starting from brains of newborn rats on account of the anti-CV2 human sera, which allowed each purification step to be monitored.

20 The tissues, preserved at -70°C before use, were treated with a solution containing 0.2 M DTT (dithiothreitol) (Sigma) 2% Ampholine 3-10 (Pharmacia), 2% Triton X-100 (Merck) and placed at 2-4°C. Immediately before use, solid urea (Pharmacia) was added to obtain an 8M solution.

25 The POP-66 protein is soluble, at least in part, and precipitates entirely at a concentration of 40% ammonium sulphate.

30 Centrifugation at 100,000 (times) g and ammonium sulphate precipitation (eliminating the proteins precipitating below 20% and above 40% ammonium sulphate) allows protein extracts enriched in POP-66 to be obtained. The proteins of this extract are then separated, after dialysis, by isofocussing on agarose gel (Peltre et al., 1982).

35 After transfer to a membrane, the anti-CV2 antibodies recognize several bands of isoelectric points of between 5.85 and 6.55. All of these bands correspond to the POP-66 protein recognized by the anti-CV2 antibodies. This spectrum suggests the

- 20 -

possibility of transcriptional modifications (phosphorylations and/or glycosylations) of the protein.

The zone of proteins of pI between 5.85 and 6.55 from the agarose gel is used for a new electrophoretic migration in denaturing medium on polyacrylamide gel previously equilibrated with an equilibration solution (0.05 mol/l Tris/HCl, pH 6.8, 6M urea, 30% glycerol, 1% weight/volume SDS for 2 x 10 minutes) to which is added DTT (0.25% weight/volume) and bromophenol blue.

Two methods of detection are used:

- *silver staining*. Immediately after the end of the migration, the gel is immersed in a fixing solution (40% ethanol, 10% acetic acid) for 30 minutes; it is then placed in an incubation solution (30% ethanol, 7% weight/volume of sodium acetate, 0.1% glutaraldehyde, 0.2% weight/volume of sodium thiosulphate) for 30 minutes or one night. After washing, the gel is placed in a silver solution (0.1% weight/volume of silver nitrate + formaldehyde) and developed (2.5% weight/volume of sodium carbonate + formaldehyde). The reaction is stopped with Na₂ EDTA (1.5% weight/volume). The gels are preserved in a glycerol solution.

- *transfer to a PVDF membrane* (Immobilon-P®, Millipore). The separated proteins are transferred to a PVDF membrane using a 100 mM CAPS buffer (Sigma) of pH 11. The transfers are incubated for one hour in TBS buffer (Tris buffer saline) with 5% of casein (milk) and 18 hours in TBS buffer (+ 1% of casein) containing antibody (1/500 anti-CV2 serum). After washing with TBS-casein (15 minutes), visualization is carried out by incubating the transfers for 1 and a half hours with biotinylated anti-IgG antibodies (1/1000) and for 1 and a half hours with the streptavidin-peroxidase complex (1/2000). The transfers are then visualized with DAB (0.06% weight/volume diaminobenzidine in 0.05 M Tris) and with H₂O₂ (0.02 µg/ml).

- 21 -

A single band corresponding to a protein of 66 kDa is visible. This is specifically labelled with anti-CV2 antibodies (Figure 1). An N-terminal sequencing of this protein was then carried out, after
5 tryptic digestion.

Seven peptides, having the following sequences, were obtained:

- 1 - X-Met-Tyr-Asp-Gly-Pro
- 2 - X-Phe-Asn-Leu-Tyr-Pro-Arg
- 10 3 - X-Val-Leu-Glu-Asp-Gly-Thr-Leu-His-Val-Thr-Glu-Gly
- 4 - X-Ile-Gly-X-X-Ala-Gln-Val-(His ?)-Ala-Glu-Asn-Gly-X-Ile-Ile-Ala-Glu-Glu-Gln
- 5 - X-X-Glu-Asn-Gln-Phe-Val-Ala-Val-Thr
- 6 - X-Val-Asn-Asp-(Asp ?)-Gln-Ser-Phe-Tyr-Ala-Asp-Ile-
- 15 Tyr-Met-Glu-(Asp ?)-(Gly ?)-Leu-Ile
- 7 - X-X-X-Phe-Val-Thr-X-Pro-X-Leu-X-Pro

X: corresponds to a non-determined amino acid,

(?): corresponds to a probable but uncertain amino acid.

20 According to the analysis of databanks available in 1994, no known protein corresponded to these sequences.

EXAMPLE 2

25 Cloning of the cDNA of POP-66 or of related proteins

The cloning of the cDNA of the POP-66 protein or of related proteins was carried out by using degenerate oligonucleotide probes obtained from fragments of two peptides:

30 Ile-Ile-Ala-Glu-Glu-Gln
Tyr-Ala-Asp-Ile-Tyr-Met-Glu-(Asp ?)

Four sets of degenerate oligonucleotide primers (sense/antisense) are therefore determined

(AT(C/T)ATTGC(T/A)GA(A/G)CA;TG(C/T)TC(T/C)AC(T/A) -
35 GCAT(A/G)AT;

TATGC(A/T)GA(C/T)AT(C/T)ATGGA; TCCAT(G/A)TA(G/A)CT-(T/A)GCATA, and used for a PCR amplification.

The matrix is prepared in the form of double-stranded cDNA (Promega kit) from poly(A⁺)RNA extracted

- 22 -

from the brain of rats 10 days old (Zivic-Miller, USA) using the Fast Track mRNA isolation kit (Invitrogen).

The conditions of amplification by PCR are as follows: 35 cycles at 94°C, 1 minute for denaturation,
5 55°C, 1 minute for hybridization and 72°C, two minutes, for extension.

The PCR products are analysed by 1% agarose gel electrophoresis, electroeluted, cloned in a TA cloning vector (Invitrogen) and sequenced using the primer
10 sites of the T7 and SP6 promoters.

The sequence of amino acids inferred from the MFB-17 clone agrees with the sequences of the two original peptides of POP-66 determined by the analysis of the amino acid sequence.

15 A comparative analysis of the nucleic acid sequences using the Genbank and EMBL databases reveals that MFB-17 is a partial cDNA with a nucleotide sequence identical to that of a segment of TOAD-64, a rat neuronal protein (Minturn et al., 1995).

20 The amino acid sequence inferred in the cDNA of TOAD-64 agrees with the sequences of the seven peptides determined by partial sequence analysis of the protein recognized by the anti-CV2 antibodies after purification by electrophoresis.

25 The molecular weight, the isoelectric point, the immunohistochemical profile and the regulation of TOAD-64 are similar to those of the POP-66 antigen.

30 Since the MFB-17 clone did not have the complete coding region, it was necessary to produce an intact recombinant protein to continue the research concerning the CV2 protein.

To obtain a complete TOAD-64 protein, the ds-cDNA matrix of rat brains was amplified with two sets of primers situated at the 5' and 3' extremities
35 of the coding regions

(sense: GGCATATGTCTTATCAGGGGAAG;

antisense: GCGAATTCTTAGCCCAGGCTGATG).

- 23 -

This approach allowed two different clones to be produced, one corresponding to the TOAD-64 sequence and the other to a clone designated by C-22.

EXAMPLE 3:

5 Comparison of the amino acid sequence inferred from C-22 with the ULIP proteins

 The amino acid sequence inferred from the open reading frame indicates that this C-22 clone belongs to the superfamily of ULIP genes represented by several
10 genes of EST sequences.

 The amino acid sequence inferred from C-22 has a homology of 30% with the amino acid sequence of the unc-33 protein of *Caenorhabditis elegans*.

 Recently, four different homologous genes in
15 the unc-33 protein have been described in mammals and the chicken.

 An analysis of the sequences by the Genbank databases and protein banks has allowed a classification of the unc-33- like (ULIP) proteins into
20 four different subgroups to be proposed (Byk et al. 1996).

 However, as the real functions of these proteins are not clearly known, the proposed classification is simply based on the percentage of
25 identity of amino acids. ULIP-1 is represented by a mouse "unc-33-like" phosphoprotein which has a homology of 76% with TOAD-64, Crmp-62 and Munc, a mouse sequence recently available from Genbank.

 ULIP-2 is composed of TOAD-64, Crmp-62 and Munc
30 which between them have a 97% amino acid identity.

 The partial human EST sequences, that is to say hcrmp-1, which have a 75% identity with ULIP-1 or ULIP-2, have been found. They belong to a third group called ULIP-3. The last group identified called ULIP-4
35 comprises r-CRMP-3 in the rat and the forms ULIP-4 in the mouse and POP-66/ULIP-4 in man.

 The comparison of the amino acid sequence of the three ULIP genes, namely TOAD-64 in the rat, Crmp-62 in the chicken and ULIP-1 in the mouse, with

- 24 -

the amino acid sequence inferred from the open reading frame of the present C-22 clone, using the Clustal V alignment software program reveals that C-22 has an identity of 74% with ULIP-1, 77% with Crmp-62 and 76% with TOAD-64.

The nucleotide sequence C-22 has an identity of 97% with the partial sequence EST, hCrmp-1, and thus defines the third member of the ULIP-3 group. The TOAD-64, Crmp-62 and C-22 genes each code for a protein of 572 amino acids in length, whereas the amino acid sequence inferred from ULIP-1 gives a protein of 570 amino acids.

The analysis of the amino acid sequence of C-22 does not show any signal sequence or transmembrane domain suggesting that the product(s) of the C-22 gene could be localized in the cytoplasm of the cells.

Several consensus sites of phosphorylation by the kinase C protein (S/T X R/K) appear along the length of the product of the C-22 gene. These observations suggest that C-22 is a phosphoprotein and that the slight differences in the phosphorylation could dictate the activity or the role of different members of this family of proteins throughout the cell cycle.

- 25 -

Table I: Summary of proteins having a homology with the ULIPs.

Family		Species	EMBL No.
Nematode Unc-33		Nematode	Z14146
Dihydropyrimidinase	Hu DHPase	human	D78011
	Ra DHPase	rat	D63704
ULIP-1 group	Ulip	mouse	X87817
	Hu DRP3	human	D78014
	r-CRMP-1	rat	U52102
	Hu-Ulip	human	Y07818
ULIP-2 group	ULIP-2	mouse	SEQ ID No. 2
	Toad-64	rat	Z46882
	CRMP-62	chicken	U17277
	Munc	mouse	X87242
	HCRMP-2	human	U17279
	Hu DRP-2	human	D78013
	r-CRMP-4	rat	U52104
ULIP-3 group	ULIP-3	mouse	SEQ ID No. 4
	HCRMP-1	human	U17278
	rCRMP-1	rat	U52102
	C-22	rat	U52095
	Hu DRP-1	human	D78012
ULIP-4 group	ULIP-4	mouse	SEQ ID No. 6
	POP-66/ULIP-4	man	SEQ ID No. 8
	r-CRMP-3	rat	U52103

5

EXAMPLE 4:

Regulation of the expression of the C-22 gene:

The evaluation of alterations in the expression of the C-22 gene could have considerable significance for the knowledge of the functional aspects of the C-22 protein.

10

Consequently, the Applicant studied the possible regulation of the expression of the C-22 gene

- 26 -

in the course of development. The total RNA is extracted and separated by electrophoresis on 1% agarose gel and transferred to Nytran membrane (Duchemin et al. 1987). The transfers are hybridized with a C-22 coding sequence labelled with ^{32}P , a 0.5 mM phosphate buffer and 5% SDS at 65°C for 16 hours.

At the end of the hybridization, the transfers are washed successively three times with 2 x SSC, 0.1% SDS at ambient temperature, then 1 x SSC, 0.1% SDS at 65°C for 60 minutes, and exposed to X-rays.

Under the conditions used, a single band at 3.8 kb was detected representing the C-22 mRNA which is also the smallest transcript of the unc-33 family of genes of vertebrates. The size of the transcript remains the same during the pre- and post-natal periods.

The kinetics of the C-22 gene in the brain of rats in the course of development shows that the messenger is detectable in the course of the embryonic period on day E17. The quantity of C-22 transcripts increases up to day 7 post-natally then decreases rapidly from the second week after birth to a virtually undetectable level in the adult.

Around birth, a still unknown regulation signal is probably received, which increases the expression of the C-22 gene, this signal being temporarily linked to neuronal differentiation and to axonal development.

The mRNA of C-22 has not been able to be detected by Northern Blot analysis in several regions of the brain such as the frontal cortex, the midbrain and the thalamus in adults and rats more than two years old.

In addition, it has not been possible to detect the mRNA of C-22 in non-neuronal tissues, such as the heart, the lung, the liver and the kidney in one-week old rats and adult rats.

The data on the expression profile of the mRNA of C-22 suggests a decisive role of the C-22 protein in the development of the brain.

- 27 -

EXAMPLE 5:**Immunoblotting of POP-66:****A - Materials and methods**

5

• Transfection of the ULIPs in *E. coli*

The full-length cDNAs of rat ULIP-2 and ULIP-3 and mouse ULIP-1 and ULIP-4 were subcloned directionally in the *E. coli* pET-21a(+) expression vector after introduction of a 5'Nde I site and of a 3' EcoRI site by PCR, and the four constructs were resequenced. The expression of the target gene induced by IPTG was carried out according to the protocol of the manufacturer (Novagen).

• Production of anti-ULIP antibodies

Rabbit antibodies (anti-Pep3) are directed against the peptide ITGPEGHVLSRPREEVE (amino acids 217-232 of the sequence SEQ ID No. 8), synthesized in a multiple peptide synthesis apparatus using F-moc (432A Peptide Synthesizer SYNERGY, Applied Biosystems). The purity was checked by analysis of the sequence by HPLC and mass spectrometry. 1 mg of the synthetic peptide conjugated to limpet haemocyanin, in complete Freund's adjuvant, was used to immunize rabbits with a booster dose of 0.5 mg of bound peptide in complete Freund's adjuvant after 4 weeks. The anti-Pep3 antibodies recognized the four recombinant ULIP proteins expressed in *E. coli*.

Labelling with the anti-Pep3 antibodies was removed after preincubation with peptide 3. Controls with pre-immune rabbit sera were negative.

Anti-peptide 4 antibodies directed against the peptide LEDGTLHVTEGS were produced according to the same protocol.

- 28 -

B - Results

Antibodies against four of the sequenced peptides were products. Two of the sera turned out to be of particular interest.

- 5 One contains antibodies (Ac anti-pep3) which recognize several members of the ULIP family on two-dimensional electrophoresis of protein extracts of newborn rat brain (Figure 2) and on one-dimensional electrophoresis (Figure 3). On Western Blotting,
10 another antibody (Ac anti-Pep4) recognizes a single band of 66 kDA capable of corresponding to a single member of the family (Figure 3), namely ULIP-2.

EXAMPLE 6:**Immunohistochemistry**

- 15 The tissue preparations for immunohistochemistry are obtained from newborn rat brains and from post-mortem human brains, fixed at 4°C in 4% paraformaldehyde and 0.2% picric acid diluted in phosphate buffer (0.1 M, pH = 7.4), then cryoprotected.
20 Immunocytochemistry can be carried out by the indirect immunofluorescence technique. Sections of 12 µm in thickness are prepared in a cryostat and then mounted on gelatin-covered slides, treated with 0.1% Triton X100 for 2 hours in PBS buffer and 1% bovine
25 serum albumin (BSA) and incubated for 12 h with anti-CV2 serum of patients in PBS/1% BSA at ambient temperature (1/100 dilution of the serum). After several washes with PBS/1% BSA, the sections are incubated for 2 h with a rabbit anti-human antiserum
30 conjugated to fluorescein, diluted to 1% (Dakopatts) in PBS/1% BSA. After washing in PBS, the slides are examined under the microscope. The control sections are incubated either with anti-human IgG antiserum conjugated to fluorescein alone, or PBS/1% BSA alone,
35 or the patient serum alone, or finally the control serum (patients not suffering from PNS) and antibodies conjugated to fluorescein at the same dilution.

To confirm the immunofluorescence, it is possible to use indirect labelling by immunoperoxidase.

- 29 -

The frozen tissue sections fixed with paraformaldehyde are incubated with 0.3% H₂O₂ (to destroy the intrinsic peroxidase activity) and 10% normal rabbit serum (to avoid the non-specific binding of the rabbit IgG) or 1% BSA. After incubation for 12 h with patient sera diluted to 1/1000 and washing, the sections are incubated for 2 h with biotinylated rabbit anti-human IgG antiserum diluted to 1/1000 in PBS/1% BSA. The bound human IgGs are visualized by incubation with an avidin-biotin-peroxidase complex (Vectastain ABC complex, Vector) and developed with 0.05% DAB (Sigma). The control sections are obtained with sera of 15 patients without PNS according to the same protocol.

A - Localization of proteins of the ULIP family with the aid of antipeptide antibodies:

Immunohistochemical labelling was carried out on sections of newborn and adult rat brains. The antipeptide-3 antibody recognizes (an) antigen(s) present in several cell types on sections of newborn and adult rat brains (Fig. 4). Like the patient anti-CV2 serum, the anti-peptide-4 antibodies do not allow the demonstration of any antigen on sections of newborn rat brain although they specifically label a subpopulation of oligodendrocytes in adult rat brain (Fig. 4).

B - Expression of POP-66 in the course of the normal development of the brain:

Figure 5 shows that the proliferative nerve cells of the progenitor zones of the nervous system demonstrated by the accumulation of bromodeoxyuridine (BrdU) do not express POP-66 although the non-proliferative cells which correspond to the nerve cells in differentiation or in migration express it.

EXAMPLE 7:

Role of POP-66 in neuronal survival

Figure 6 allows human brain sections of healthy patients and of patients suffering from PNS to be compared. In the patients suffering from PNS and having circulating anti-CV2 antibodies, a disappearance of the

- 30 -

neurons of the dentate gyrus and of pyramidal neurons (central cell band), as well as an intense astrocytic reaction, are observed.

EXAMPLE 8:

5 Characterization of the POP-66 protein -
identification with ULIP-4:

Materials and methods

10 a) Partial purification of ULIP-1

Partially purified ULIP-1 was obtained from newborn mouse brains by three purification steps. These brains were homogenized in 4 volumes of homogenization buffer (25 mM sodium phosphate, pH 7.8, 1 mM EGTA,
15 10 µg/ml of leupeptin, 25 µg/ml of aprotinin, and 10 µg/ml of pepstatin. The homogenates were centrifuged for 10 minutes at 400 × g. The plugs were resuspended in 2 volumes of homogenization buffer, homogenized and centrifuged again. The supernatants from two
20 centrifugations were collected, sonicated and centrifuged for 1 hour at 100,000 × g. The supernatant (S2) was loaded onto a column of DEAE-Sepharose CL-6B (1.75 cm² × 26 cm) equilibrated with 100 ml of buffer A (25 mM sodium phosphate, pH 7.8, 1 mM EGTA) at a flow
25 of 30 ml per hour. The proteins were eluted in 300 ml of a 0-250 mM linear gradient of sodium chloride in buffer A and 5 ml samples were collected. The fractions containing ULIP were collected and solid ammonium sulphate was added to 20% saturation. This pool was
30 loaded onto a column of phenyl-Sepharose CL-4B (1.75 cm² × 22 cm) which had been previously equilibrated with 100 ml of buffer B (10 mM sodium phosphate, pH 7.8, 1 mM EGTA) containing 20% of saturated ammonium sulphate. The proteins were eluted
35 in a linear gradient decreasing from 20 to 0% of saturated ammonium sulphate in buffer B. The fractions containing ULIP were collected and dialysed twice against 20 volumes of buffer A. The proteins were concentrated in a small (10 ml) column of DEAE-

- 31 -

Sepharose CL-6B and eluted with 400 mM sodium chloride in buffer A. The eluate was desalted on a Sephadex G-25 (NAP-10) column and concentrated to a final volume of 0.5 ml by evaporation. In the last purification step, the concentrated fraction was chromatographed in three successive steps, on two Superose 12 FPLC (Fast Protein Liquid Chromatography) columns mounted in series, in buffer C (50 mM sodium phosphate, pH 7.2, 150 mM sodium chloride) at a rate of 0.3 ml/minute. The fractions (0.6 ml) were collected and the fractions enriched in ULIP were analysed. The presence of ULIP in the successive purification steps was tested by a one-dimensional Western Blot using an anti-stathmin antibody capable of cross-reactivity. The proteins were quantified according to the method of Bradford.

b) Migration on electrophoresis gel:

A one-dimensional electrophoresis was carried out on 13% polyacrylamide gels according to the method of Laemmli. The two-dimensional PAGE electrophoreses were carried out as described above. The isoelectrofocussing gels contained 2% of total ampholines, pH 6-8 and 3-10 in a ratio of 4:1. The second dimension had been carried out on 10% acrylamide gels. The proteins had been either subjected to immunoblotting or stained with silver.

c) Western Blot analysis:

The proteins were transferred from gels to nitrocellulose in buffer containing 48 mM Tris, 39 mM glycine and 5% of methanol. The membrane was saturated with casein (2.5%) in the immunoblotting solution (12 mM Tris-HCl, pH 7.4, 160 mM NaCl, 0.1% Triton X-100) and tested with an antiserum directed against the peptide I of rat stathmin (1/10,000 dilution) or an antiserum directed against the recombinant ULIP protein (dilution 1/20,000) diluted in an immunoblotting solution containing 1% of casein. The bound antibodies were detected either with a protein A labelled with ^{125}I and autoradiographed or with anti-rabbit antibodies bound to peroxidase using the ECL kit (Amersham).

- 32 -

d) Analysis of the protein sequence:

The fractions enriched in ULIP were separated on polyacrylamide gels in two dimensions. The gels are fixed for 30 minutes in 25% ethanol and 10% acetic acid and stained for 3 minutes in 0.1% amido black in 1% acetic acid and 40% methanol. The gels were decolourized in 1% acetic acid and the spots corresponding to the principal form of ULIP were cut out in these three gels, collected and digested with 2 mg/ml of Lys C endoprotease. The peptides eluted from the gel were then separated by HPLC on a DEAE-C18 column with a gradient of 0-55% of acetonitrile in 0.1% trifluoro-acetic acid. The peptides were then sequenced according to the Edman automatic degradation.

e) Expression in vitro in a mammal

1 µg of the Bluescript plasmid containing the entire cDNA coding for ULIP-1, ULIP-2, ULIP-3 or ULIP-4 was used to carry out the transcription and translation in vitro with the "Reticulocyte lysate" system (Promega) according to the protocol described by the manufacturer. 5 µg of the total transcription/translation mixture of 25 µl were analysed on electrophoresis gel in two dimensions.

Results

Neither the recombinant protein ULIP-1, nor the recombinant proteins TOAD-64 (ULIP-2) and C-22 (ULIP-3) were recognized by the anti-CV2 sera. In addition, the distribution profile of the spots corresponding to POP-66 recognized by the anti-CV2 antibodies on electrophoresis in two dimensions does not correspond to the spots recognized by the anti-ULIP-1 antibodies. However, POP-66 is a member of the ULIP family since the three POP-66 spots are recognized by the anti-pep3 Ac. POP-66 therefore corresponds to a member of the family of more basic pH_i.

After translation in vitro of the four proteins (ULIP-1, 2, 3 and 4), it was shown that ULIP-4 has the same 2D electrophoretic profile as POP-66 and is recognized by the anti-CV2 antibodies (Figure 7).

- 33 -

For this, the ULIP-4 protein and, as control, the ULIP-2 protein were translated *in vitro* in the presence of ^{35}S methionine from cDNA clones coding for the entire proteins. The proteins were separated by two-dimensional electrophoresis (in the presence of a brain extract providing the essential references), transferred to nitrocellulose and visualized:

- by autoradiography to localize the proteins translated *in vitro* (translation);

- by immunoblotting with the CV2 serum.

Figure 7 shows that the three spots from the *in vitro* translation of ULIP-4 correspond to the spots recognized by CV2. These spots are not recognized in the translation of ULIP-2.

The CV2 serum therefore specifically recognizes ULIP-4.

This allowed POP-66 to be identified like ULIP-4.

EXAMPLE 9:

Chromosomal localization of the POP-66/ULIP-4 protein

Having cloned the cDNA of human ULIP-4, it is then possible to determine the chromosomal localization of the POP-66/ULIP-4 gene by genetic mapping by *in situ* isotopic hybridization (Levy and Mattei et al., 1995).

In situ hybridization is carried out on preparations of chromosomes obtained from human lymphocytes stimulated by phytohaemagglutinin cultured for 72 hours. 5-Bromodeoxyuridine was added during the last 7 hours of culture (60 $\mu\text{g}/\text{ml}$ of medium) to ensure a post-hybridization image of chromosome bands of good quality. The clone containing an insert of 1300 base pairs coding for ULIP-4 in the Bluescript vector is labelled with tritium by nick translation with a specific activity of 1×10^8 dpm. μg^{-1} . The radiolabelled probe was hybridized in the metaphase stage at a final concentration of 200 ng per ml of hybridization solution. After covering with a Kodak NTB₂ emulsion, the slides were exposed for 20 days at

- 34 -

+4°C and then developed. To avoid the shifting of the silver grains during the process, the chromosome blots were previously labelled with a Giemsa buffer solution and the metaphases were photographed. The visualization of the bands was carried out by the "Giemsa fluorochrome photolysis" (FPG) method and the metaphases were rephotographed before analysis. Of the 100 cells in metaphase examined after hybridization *in situ*, 246 silver grains associated with the chromosomes were counted and 54 amongst these (21.9%) were localized on chromosome 10. The distribution of the grains on this chromosome was not random: 39 out of 54 (72.2% of the latter) were localized on the q25.2-q26 region of the long arm of chromosome 10.

The POP-66/ULIP-4 gene is therefore found to be situated on chromosome 10 in the q25.2-q26 region. The loci of neurodegenerative diseases and of suppressor genes of tumours involved in different types of cancer have been localized in this chromosome region. The locus of a brain disease of early origin (infantile onset spinocerebellar ataxia) was identified in the 10q24-26 region (Varilo et al., 1996; Nikali et al., 1995). The symptoms of this recessive hereditary degenerative disease which is characterized by ataxia, neuropathy and visual atrophy are similar to those observed in patients suffering from paraneoplastic neurological syndromes with circulating anti-CV2 auto-antibodies (Honnorat et al., 1996). On the other hand, 80% of glioblastomas have mutations in this chromosome region and several suppressor loci involved in different types of tumours (prostate, kidney, small-cell lung cancer and endometrial carcinomas) are localized in this chromosome region. These data support the possibility that POP-66/ULIP-4 plays a crucial role in neurodegeneration and tumorigenesis.

In this respect, it is notable that the expression of ULIP-1 is regulated upwards in neuroblastoma cells differentiated by retinoic acid and that ULIP-1 and ULIP-3 are regulated upwards but ULIP-4

- 35 -

is regulated downwards in differentiated PC12 cells in the presence of NGF, suggesting that the stop in cell growth can be linked to expression levels of the ULIP proteins.

5

EXAMPLE 10

Expression of ULIP proteins in transfected HeLa cells

A- Materials and methods

10

A flag (EcoRI-ATGGACTACAAGGACGACGATGACAAGG-BamHI) sequence (Kodak) was cloned in the EcoRI site of pSG5 followed by ULIP-1 (EMBEL X87817), base pairs: 309-2023), ULIP-2 (Y10339, base pairs: 23-1741), ULIP-3 (Y09080, base pairs: 269-1991) or ULIP-4 (Y09079, base pairs: 102-1820), respectively. The HeLa cells were cultured in DMEM media (Gibco) to which 10% of foetal calf serum (v/v) was added. The transfections were carried out by calcium phosphate precipitation (Maniatis et al., 1978). The HeLa cells were mixed with 5 µg of pSG5flag-ULIP-1, 2, 3 and 4 plasmids and 10 µg of pUC18. Twenty-four hours after the transfection, the HeLa cells were fixed with 4% paraformaldehyde and immunolabelled with different human sera (dilution 1/300), visualized by human anti-IgG antibodies conjugated to FITC (Biosys), or anti-flag antibodies (M2, Kodak) (dilution 1/1000), visualized by anti-rabbit antibodies conjugated to Texas red (Vector).

Double immunolabelling was carried out on the HeLa cells transfected with ULIPs using anti-flag and anti-Pep3 antibodies. In the cells transfected by any cDNA, 10 to 20% among them showed immunolabelling with the anti-flag antibodies visualized by the anti-mouse antibodies conjugated to Texas red.

All the transfected cells were doubly labelled by antibodies directed against Pep3 and a peptide common to the four ULIPs is visualized by rabbit anti-IgG antibodies conjugated to fluorescein.

Double immunolabelling was likewise carried out on HeLa cells transfected with ULIPs using anti-flag

- 36 -

and anti-CV2 antibodies. The human sera of patients suffering from PNS with circulating anti-CV2 auto-antibodies labelled the cells transfected by ULIP-4, and an anti-CV2 serum likewise labelled the cells transfected by ULIP-3. No labelling of the cells transfected by ULIP-4 was detected in the control sera of patients without cancer or neurological disease.

B) Results

After transfection of HeLa cells with cDNAs labelled by the flags of ULIP-4, 10 to 20% of the cells were strongly reactive with anti-flag antibodies and anti-Pep3 antibodies which recognize the ULIP-4s of mammals. The transfected cells were not immunolabelled with control serum of 10 neurological patients without PNS nor with rabbit pre-immune serum. On the other hand, the cells transfected with small cDNA of ULIP-4 showed an intense immunoreactivity with all the 7 tested sera of patients with circulating anti-CV2 auto-antibodies. These sera are negative on cells transfected with cDNAs of other ULIPs, with the exception of a sera which also recognized the cells transfected with ULIP-3 and a serum which also recognized the cells transfected with ULIP-1, 3 and 4. No labelling was observed on non-transfected HeLa cells, with an anti-CV2 serum.

Table 1 below shows the results of indirect immunofluorescence with different sera on HeLa cells by labelled cDNAs of members of the ULIP family.

- 37 -

Table 1:

Serum No.	Neurological Symptoms	Type of Tumour	ULIP-1	ULIP-2	ULIP-3	ULIP-4
Anti-Pep3	-	-	+	+	+	+
Pre-immune	-	-	-	-	-	-
Pep3						
90-002	PCD, uveitis	UC	-	-	+	+
93-484	LE	Thymoma	-	-	-	+
94-590	LE	SCLC	-	-	-	+
95-700	PEM	SCLC	+	-	+	+
95-701	PCD	Uterine sarcoma	-	-	-	+
95-706	LE, neuropathy	SCLC	-	-	-	+
97-040	PCD	SCLC	-	-	-	+
97-103	PCD	SCLC	-	-	-	+

PCD: paraneoplastic cerebellar degeneration;

5 LE: lymbic encephalitis;

PEM: paraneoplastic encephalomyelitis;

UC: undifferentiated carcinoma;

SCLC: small-cell lung carcinoma.

10 EXAMPLE 11:

Expression of POP-66/ULIP-4 and of members of
the ULIP family in cancers

15 A - Expression of ULIP-2 and ULIP-3 in cancers:

1) Materials and methods: RT-PCR experiments:

The total RNA was extracted using 1 ml of
RNAZOL™B (Bioprobe) according to the method of
Chomczynski and Sacchi. The quantity of RNA was
20 determined by optical density measured at 260 nm and
its purity was determined from the ratio of the
absorbances measured at 260 and 280 nm (ratios 1.8-
2.0). The integrity of the RNA preparations was
25 additionally checked by electrophoresis on 1% agarose
gel in TBE (0.45 M Tris-borate, 10 mM EDTA, pH 8). The

- 38 -

specificity of the primers was analysed by comparing their sequences with the various gene databanks (EMBL and FASTA). For a relative quantification, the gene coding for G3PDH (glyceraldehyde-3-phosphate dehydrogenase, Clontech), a ubiquitous gene expressed in numerous tissues including the brain, was co-amplified with the mRNA tested as an internal standard to check the uniformity of the quantities of RNA in the samples and to test the efficacy of the reverse transcription step for different RNA samples. The primers 5', 3' and the oligonucleotides of internal probes of G3PDH were synthesized and purified by Eurogentec. The total mRNA (1 µg) was denatured (15 minutes at 65°C) and transcribed in single-stranded cDNA (1 and a half hours, 42°C) in a final volume of 20 µl of buffer (50 mM Tris HCl, 75 mM KCl, pH 8.3, Gibco BRL) containing 5 ng per µl of oligo-dT 12-18 primer (Pharmacia Biotech), 40 units of reverse transcriptase of the Moloney murine leukaemia virus (Mu-LV) (Gibco BRL), 40 units of RNAsine (Promega), 10 mM DTT (Gibco BRL) and 0.5 mM of each of the triphosphate deoxynucleotides (Promega). The cDNA samples were diluted to 1/10 in distilled water and the PCR reactions were carried out using 1 µl, 4 µl or 2 µl of cDNA sample for the messenger RNA of ULIP-2 and ULIP-3, in a buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X100, 0.4% glycerol and 800 µM NaCl, pH 9), to which was added 40 µmol of DTT, 3 mmol of MgCl₂, 0.2 mmol of each dNTP, 0.4 µM of each selected primer and 2 units of AmpliTaq DNA polymerase (Promega) in a final volume of 50 µl. The samples were then placed in a thermocycler (Biomed-Hybaid), denatured at 95°C for 5 minutes and amplified for 35 cycles (one cycle = 95°C denaturation for 65 seconds, 60°C hybridization of the primers for 45 seconds, 72°C extension for 4 minutes and 15 minutes final elongation at 72°C. The products were separated by electrophoresis on 1% Seakem agarose gel and the test bands of the RT-PCR products of expected size as well as the molecular weight marker

- 39 -

scale (100 base pairs) (Promega) were visualized using ethidium bromide staining.

Composition of the oligonucleotide probes used for ULIP-3 PCR

5 5' ATAGAGGAGCGGATGACG (899) 3'

GCTGTTATGGTCTTCAACTTGTCGG (1092)

GGCCTGTTATGGTCTTCAACTTGTCG (1093)

Composition of the oligonucleotide probes used for ULIP-2 PCR

10 5' AGGAGGAGTGAAGACCATCG 5227) 3'

CTTATGCCACTCGCTGATGTCC (509).

2) Results

The RT-PCR experiments show that TOAD-64 (ULIP-2) and C-22 (ULIP-3) are expressed in certain small-cell lung tumours (cf. Figure 8) and absent in others, especially in cells of patients who develop paraneoplastic neurological syndromes with a good prognosis.

B - Expression of ULIP-4 in cancers

1) Materials and methods

25 • Preparation of the RNA and RT-PCR

The total RNAs are extracted from cerebral tumours preserved in liquid nitrogen according to the conventional RNAZOL™ technique (Bioprobe, France). Reverse transcription was carried out using oligo(dt)₁₈ on 1 µg of total RNA and the PCR was carried out with 1/20 of the volume of the mixture for the reverse transcription (RT mix). The primers used for ULIP-4 are:

35 5'CATCTGGCTGTCGCTGCAC3', 5'GCCGCCCTACCAGAGACC3',
and for GAPDH: 5'GGAGATTTCAGTGTGGTGG3',
5'GGCTCTCCAGAACATCATCC3'. The cDNA was denatured at 95°C for five minutes. PCR amplification was carried out for 30 cycles. ULIP-4: 95°C, 45 sec; 62°C, 45 sec; 72°C, 45 sec. GAPDH: 95°C, 45 sec; 55°C, 45 sec; 72°C,

- 40 -

45 sec. The final extension was carried out at 72°C for 5 minutes.

2) Results

5 Of the 8 glioblastoma extracts studied, 4 (50%) expressed the messenger RNA of ULIP-4. Conversely, of the 10 oligodendroglioma extracts tested, none expressed the messenger RNA of ULIP-4. This differential expression, as a function of the primitive
10 cerebral tumour type, is in favour of a potential role of ULIP-4 in the cell proliferation of these tumours.

The protein POP-66/ULIP-4 as well as the proteins of the ULIP family could be expressed in the peripheral tumours (small-cell lung tumour, thymoma,
15 cancer of the breast and of the ovary). Their presence could therefore be correlated with a prognosis. The localization of the POP-66/ULIP-4 gene on the distal part of chromosome 10 confirms this in the case of cerebral tumours.

20 Thus, the differential expression of members of the ULIP family in tumours such as small-cell lung cancer, although the corresponding ULIP gene is absent in a healthy tissue, as well as the modulation of the expression of members of the ULIP family obtained
25 during differentiation by the HTLV1 human retrovirus of a medulloblastoma line, suggest the involvement of ULIPs in cancerous tumours.

EXAMPLE 12:

30 Production of specific antibodies of each of the human ULIP proteins

Specific peptides of each member of the ULIP family were synthesized on a multiple peptide synthesis apparatus using F-moc (432A Peptide Synthesizer
35 SYNERGY, Applied Biosystems). The purity was checked by sequence analysis by HPLC and mass spectrometry.

These peptides are:

Specific peptide of ULIP-1: G S A R G S P T R P N (11 amino acids)

- 41 -

Specific peptide of ULIP-2: S S A K T S P A K Q Q A (12 amino acids)

Specific peptide of ULIP-3: P S A K S S P S K H Q (11 amino acids)

5 Specific peptide of ULIP-4: P A R A S C P G K I S (11 amino acids).

1 mg of the synthetic peptide conjugated to limpet haemocyanin, in complete Freund's adjuvant, was used to immunize rabbits with a booster dose of 0.5 mg of bound peptide in complete Freund's adjuvant after 4 weeks.

The antibodies obtained specifically recognize each member protein of the ULIP family.

15

EXAMPLE 13:

Production of transgenic animals expressing ULIP-4

Drosophila fruit flies were transformed by the cDNA of human ULIP-4.

20

The cDNA of ULIP-4, previously cloned in pbluescript SK-phagemid, was excised by KpnI and XbaI enzymatic double digestion. After electrophoresis on agarose gel, the cDNA fragment was purified and then cloned in pUAST, originating from pCaSpeR3, digested by the restriction enzymes KpnI and XbaI. The 10-C plasmid results from the directional cloning of the cDNA of ULIP-4 in pUAST associated with the mini-white reporter gene. The 10-C plasmid was injected with a p-delta-2-3 helper plasmid coding for the transposase of the P element active in the germinal line.

25

30

The transformed fruit flies are identified by their red eyes resulting from the expression of the mini-white gene. These lines transformed by the cDNA of ULIP-4 under the control of UASGAL4 regulatory sequences allow a targeted expression of the cDNA of ULIP-4.

35

This production of transformed fruit flies allows the role of ULIP-4 to be studied specifically in

- 42 -

different cells and its involvement in human pathologies to be understood.

17-AUG-1999 13:23 FROM RWS TRANSLATIONS LTD
TO 90033148745456
P.18

- 43 -

BIBLIOGRAPHY

- 5 - Anderson et al., CRC Crit. Rev. Neurobiol., 1987, vol. 3, pp 245-99
- Antoine J.C. et al., Journal of the Neurological Sciences, 1993, vol. 117, pp 215-223
- Byk et al., Journal of Neuroscience, 1996, vol. 16(2), pp 688-701
- 10 - Chomczynsky and Sacchi, Anal. Biochem., 1987, 162: 156-159
- Dalmau et al., Neurology, 1991, vol. 41, pp 1757-64
- Duchemin et al., Dev Neurosci, 1987, vol. 9, pp 61-67
- Graus et al., Neurology, 1985, vol. 35, pp 538-543
- 15 - Graus et al., Neurology, 1990, vol. 40, pp 219-22
- Greenlee et al., Ann. Neurol., 1983, vol. 14, pp 609-13
- Hamajima et al., Gene, 1996, vol. 180, pp 157-163
- Hetzel et al., Mayo Clin. Proc., 1990, vol. 65, pp 1558-63
- 20 - Honnorat J. et al., Journal of Neurology, Neurosurgery and Psychiatry, 1996, vol. 61, pp 270-278
- Jaekle et al., Ann. Neurol., 1985, vol. 18, pp 592-600
- 25 - Köhler and Milstein, Nature, 1975, vol. 256, pp 495-497
- Levy N., Mattei MG., 1995, Geneprobs II. A practical approach. BD Hames and SJ Higgins, Oxford University Press, pp 211-243
- 30 - Luque et al., Ann. Neurol., 1991, vol. 29, pp 241-51
- Minturn et al., J. Neurosci., 1995, vol. 15, pp 6757-6766
- Nikali et al., Am. J. Hum. Genet., 1995, 56, 1088-1095
- 35 - Peltre G., Lapeyre J.; David B., Immunol. Lett., 1982, vol. 5, pp 127-131
- Sambrook et al., Molecular Cloning, a laboratory Manual, 1989, 9.47-9.62

- 44 -

- Varilo et al., Genome Res., 1996, 6:870-875
- Wang L-H et al., J. Neurosci., 1996, vol. 16(9),
pp 6197-6207

- 45 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

(A) NAME: Institut National de la Santé et
de la Recherche Médicale

(B) STREET: 101, rue de Tolbiac

(C) CITY: Paris

10

(E) COUNTRY: FRANCE

(F) POSTAL CODE: 75013

(G) TELEPHONE: 0144236000

(H) TELEFAX: 0145856856

15

(ii) TITLE OF INVENTION: Use of ULIP in PNS and
associated cancers

(iii) NUMBER OF SEQUENCES: 8

20

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

25

(D) SOFTWARE: PatentIn Release #1.0,
Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1817 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

- 46 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTTCCTCCCG	CCCCCGGAG	AGATGTCTTA	TCAGGGGAAG	AAAAATATTC	CACCCATCAC	60
GAGCGATCGT	CTTCTGATCA	AAGGTGGCAA	GATTGTGAAT	GATGACCACT	CCTTCTATGC	120
AGACATATAC	ATGGAAGATG	GGTTGATCAA	GCAAATAGGA	GAAAACCTGA	TTGTACCAGG	180
AGGGGTGAAG	ACCATCGAAG	CCCACTCCAG	AATGGTGATT	CCCGGAGGAA	TTGACGTGCA	240
TACTCGCTTC	CAGATGCCTG	ACCAGGGAAT	GACATCCGCT	GATGACTTCT	TCCAGGGAAC	300
CAAGGCGGCC	CTGGCCGGGG	GAACCACCAT	GATCATTGAC	CATGTTGTTC	CTGAGCCCGG	360
GACGAGCCTA	TTGGCTGCCT	TTGATCAGTG	GAGGGAGTGG	GCTGACAGCA	AGTCCTGCTG	420
TGACTATTCT	CTGCACGTGG	ACATCACTGA	GTGGCAACAAG	GGCATCCAGG	AGGAGATGGA	480
AGCTCTGGTG	AAGGACCACG	GGGTAAACTC	CTTCCTCGTG	TACATGGCTT	TCAAAGATCG	540
ATTCCAGCTG	ACGGATTCCC	AGATCTATGA	AGTCTGAGC	GTGATCCGGG	ATATCGGTGC	600
CATAGCTCAA	GTCCACGCAG	AGAATGGTGA	CATCATTGCT	GAGGCACAGC	AGAGGATCCT	660
GGATCTGGGC	ATCACGGGCC	CCGAGGGACA	CGTGTGAGC	CGGCCAGAGG	AGSTCGAGGC	720
TGAAGCTGTG	AACCGGTCCA	TCATATTGTC	CAACCAGACC	AACTGCCCTC	TGTATGTCAC	780
CAAAGTGATG	CCCAAGAGTG	CGGCTGAAGT	CATCGCTCAG	GCACGGAAGA	AGGGAAGTGT	840
GGTGTATGGT	GAGCCCATCA	CGGCCAGCCT	GGGGACTGAT	GGCTCTCATT	ACTGGAGCAA	900
GAAGTGGGCC	AAGGCTGCGG	CCTTTGTGAC	CTCCCCACCC	TTGAGCCCCG	ACCCAACCAC	960
TCCAGACTTT	CTCAACTCGT	TGCTGTCTTG	TGGAGACCTC	CAAGTCACTG	GCAGTGCCCCA	1020
CTGCACCTTC	AACACTGCCC	AGAAGGCTGT	GGGGAAGGAC	AACTTCACCT	TGATTCCCGA	1080
GGGCACCAAC	GGCACTGAGG	AGCGGATGTC	TGTCATTTGG	GATAAAGCTG	TGGTCACTGG	1140
GAAGATGGAT	GAGAATCAGT	TTGTGGCTGT	GACCAGCACC	AACGCAGCCA	AAGTCTTCAA	1200
CCTTTACCCC	CGGAAAGGTC	GCATCTCGGT	GGGATCTGAT	GCTGACTTGG	TCATCTGGGA	1260
CCCTGACAGT	GTGAAGACCA	TCTCTGCCAA	GACACACAAC	AGTGCTCTTG	AGTACAACAT	1320
CTTTGAAGGC	ATGGAGTGTC	CCGGCTCCCC	ACTGGTGGTC	ATCAGCCAGG	GCAAGATTGT	1380
CCTGGAGGAC	GGCACACTTC	ATGTCACTGA	AGGCTCAGGA	CGCTACATTC	CCCGGAAGCC	1440
CTTCCTGAC	TTTGTGTACA	AACGCATCAA	AGCAAGGAGC	AGGCTGGCTG	AGCTGAGAGG	1500
GGTCCCTCGT	GGCCTGTATG	ACGGACCGGT	ATGCGAGGTG	TCTGTGACGC	CCAAGACGGT	1560
GACTCCAGCC	TCATCAGCTA	AGACATCCCC	TGCCAAGCAG	CAGGCACCAC	CTGTTCCGAA	1620
CCTGCACCAG	TCTGGATTCA	GCTTGTCTGG	TGCTCAGATT	GACGACAACA	TTCCCCGCCG	1680
CACCACCCAG	CGCATCGTGG	CACCCCTGGG	TGGCCGTGCC	AACATCACCA	GCCTGGGCTA	1740
AAGCCCTAG	GCCTGCAGGC	CACTTGGGGA	TGGGGGATGG	GACACCTGAG	GACATTCTGA	1800
GACTTCCTTT	CTTCCAT					1817

- 47 -

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Tyr Gln Gly Lys Lys Asn Ile Pro Pro Ile Thr Ser Asp Arg
 1 5 10 15
 Leu Leu Ile Lys Gly Gly Lys Ile Val Asn Asp Asp Gln Ser Phe Tyr
 20 25 30
 Ala Asp Ile Tyr Met Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
 35 40 45
 Leu Ile Val Pro Gly Gly Val Lys Thr Ile Glu Ala His Ser Arg Met
 50 55 60
 Val Ile Pro Gly Gly Ile Asp Val His Thr Arg Phe Gln Met Pro Asp
 65 70 75 80
 Gln Gly Met Thr Ser Ala Asp Asp Phe Phe Gln Gly Thr Lys Ala Ala
 85 90 95
 Leu Ala Gly Gly Thr Thr Met Ile Ile Asp His Val Val Pro Glu Pro
 100 105 110
 Gly Thr Ser Leu Leu Ala Ala Phe Asp Gln Trp Arg Glu Trp Ala Asp
 115 120 125
 Ser Lys Ser Cys Cys Asp Tyr Ser Leu His Val Asp Ile Thr Glu Trp
 130 135 140
 His Lys Gly Ile Gln Glu Glu Met Glu Ala Leu Val Lys Asp His Gly
 145 150 155 160
 Val Asn Ser Phe Leu Val Tyr Met Ala Phe Lys Asp Arg Phe Gln Leu
 165 170 175
 Thr Asp Ser Gln Ile Tyr Glu Val Leu Ser Val Ile Arg Asp Ile Gly
 180 185 190
 Ala Ile Ala Gln Val His Ala Glu Asn Gly Asp Ile Ile Ala Glu Ala
 195 200 205

- 48 -

Gln Gln Arg Ile Leu Asp Leu Gly Ile Thr Gly Pro Glu Gly His Val
210 215 220
Leu Ser Arg Pro Glu Glu Val Glu Ala Glu Ala Val Asn Arg Ser Ile
225 230 235 240
Thr Ile Ala Asn Gln Thr Asn Cys Pro Leu Tyr Val Thr Lys Val Met
245 250 255
Pro Lys Ser Ala Ala Glu Val Ile Ala Gln Ala Arg Lys Lys Gly Thr
260 265 270
Val Val Tyr Gly Glu Pro Ile Thr Ala Ser Leu Gly Thr Asp Gly Ser
275 280 285
His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Ala Phe Val Thr Ser
290 295 300
Pro Pro Leu Ser Pro Asp Pro Thr Thr Pro Asp Phe Leu Asn Ser Leu
305 310 315 320
Leu Ser Cys Gly Asp Leu Gln Val Thr Gly Ser Ala His Cys Thr Phe
325 330 335
Asn Thr Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Thr Leu Ile Pro
340 345 350
Glu Gly Thr Asn Gly Thr Glu Glu Arg Met Ser Val Ile Trp Asp Lys
355 360 365
Ala Val Val Thr Gly Lys Met Asp Glu Asn Gln Phe Val Ala Val Thr
370 375 380
Ser Thr Asn Ala Ala Lys Val Phe Asn Leu Tyr Pro Arg Lys Gly Arg
385 390 395 400
Ile Ser Val Gly Ser Asp Ala Asp Leu Val Ile Trp Asp Pro Asp Ser
405 410 415
Val Lys Thr Ile Ser Ala Lys Thr His Asn Ser Ala Leu Glu Tyr Asn
420 425 430
Ile Phe Glu Gly Met Glu Cys Arg Gly Ser Pro Leu Val Val Ile Ser
435 440 445
Gln Gly Lys Ile Val Leu Glu Asp Gly Thr Leu His Val Thr Glu Gly
450 455 460
Ser Gly Arg Tyr Ile Pro Arg Lys Pro Phe Pro Asp Phe Val Tyr Lys
465 470 475 480
Arg Ile Lys Ala Arg Ser Arg Leu Ala Glu Leu Arg Gly Val Pro Arg
485 490 495
Gly Leu Tyr Asp Gly Pro Val Cys Glu Val Ser Val Thr Pro Lys Thr
500 505 510
Val Thr Pro Ala Ser Ser Ala Lys Thr Ser Pro Ala Lys Gln Gln Ala
515 520 525
Pro Pro Val Arg Asn Leu His Gln Ser Gly Phe Ser Leu Ser Gly Ala
530 535 540
Gln Ile Asp Asp Asn Ile Pro Arg Arg Thr Thr Gln Arg Ile Val Ala
545 550 555 560
Pro Pro Gly Gly Arg Ala Asn Ile Thr Ser Leu Gly
565 570

- 49 -

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2297 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

5
10
15

GCTGTCTGTC TTCAGCGCCG TCTCTCGCC CTGCCTCTCC CTCCTCCTCC CGCCCTCCTT	60
GCCAAGCCGG GCGGTGCGG CAGCCGAGC AGCGGCGGGG GCGCGAGCAG CGGGGAGTGG	120
GCAGCGGTGG GAGCCGAGCT TCTGTCTTTT CTTTCATCCC TCCCTGGCCT TTGTCGCGGC	180
TCTCAGGAGT AGCGCCGCCC GGAGAGACCC GGGTAGAGCG CCAGGCAGAC GTTAGTTCCA	240
GCGGCCGGGG GGAGGGGCTC AGAGGGGCCA TGTCTCATCA GGGGAAGAAG AGCATCCCGC	300
ACATCACCAG TGACCGGCTC CTCATCAGAG GTGGACGCAT CATCAATGAT GACCAGTCCT	360
TCTACGCCGA TGTCTAGCTA GAAGATGGAC TCATAAAACA AATAGGAGAG AACCTGATTG	420
TTCCTGGTGG AGTGAGGACC ATCGAGGCGA ATGGCCGAAT GGTCAATCCC GGTGGCATTG	480
ATGTCAACAC TTACCTGCAG AAGCCCTCCC AGGGCATGAC CTCGGCTGAT GACTTCTTCC	540
AGGGCACTAA AGCAGCGCTG GCAGGTGGAA CCACGATGAT CATTGACCAC GTTGTTCCTG	600
AACCTGGGTC CAGCTTGTTG ACTTCCTTTG AGAAATGGCA CGAAGCAGCA GACACCAAAT	660
CCTGCTGTGA CTATTCCCTC CACGTGGACA TCACAAGCTG GTATGATGGT GTTCGGGAAG	720
AGCTGGAGGT GCTGGTGCAG GACAAAGGTG TCAACTCCTT CCAAGTCTAC ATGGCGTATA	780
AGGACCTGTA CCAGATGTCT GACAGCCAGC TGTATGAAGC CTTACCTTC CTTAAGGGTT	840
TGGGAGCTGT GATCTTAGTC CATGCAGAAA ATGGAGATTG GATAGCTCAG GAACAAAAAC	900
GGATCCTGGA GATGGGCATC ACGGGTCCCG AGGGTCATGC TCTGAGCAGA CCCGAGGAGC	960
TGGAGGCCGA GGCTGTGTTT CGGGCTATTG CCATTGCAGG CCGGATCAAT TGCCCTGTGT	1020
ACATCACCAA GGTGATGAGC AAGAGTGCAG CGGACATCAT CGCACTGGCC AGGAAGAAAG	1080
GCCCTCTTGT CTTGGGTGAG CCCATAGCCG CCAGCCTGGG AACCGATGGC ACCCACTACT	1140

- 50 -

GGAGCAAGAA CTGGGCCAAG GCAGCTGCAT TTGTGACTTC CCCTCCCTG AGCCCAGACC	1200
CCACCACTCC TGACTACTTG ACCTCCTTGC TGGCCTGTGG AGACTTGCAG GTCACAGGTA	1260
GTGGCCACTG TCCCTACAGT ATTGCTCAGA AGGCTGTGGG CAAGGACAAC TTCACTCTGA	1320
TCCCTGAGGG TGTCAATGGT ATAGAAGAGC GGATGACCGT TGTCTGGGAC AAGGCAGTGG	1380
CTACTGGCAA GATGGATGAG AACCAGTTTG TAGCCGTAC CAGCACC AAC GCAGCCAAGA	1440
TCTTCAACCT GTACCCGAGG AAAGGTCGGA TCGCTGTGG CTCCGATGCT GACGTAGTCA	1500
TCTGGGACCC AGATAAGATG AAGACCATAA CAGCCAAAG CCATAAATCA ACTGTGGAGT	1560
ACAACATCTT TGAGGGCATG GAGTGCCACG GCTCCCTTCT GGTGGTCATC AGTCAGGGCA	1620
AGATTGTCTT TGAGGATGGA AACATCAGTG TCAGCAAGGG CATGGGCCGC TTCATCCCTC	1680
GGAAGCCATT CCCAGAGCAT CTCTACCAGC GTCTCAGGAT CAGAAGCAAG GTTTTCGGGT	1740
TGCATAGTGT TTCCAGGGGC ATGTACGATG GACCTGTGTA CGAGGTGCCA GCTACACCCA	1800
AACATGCTGC TCCTGCTCCT TCTGCCGAAT CCTCGCCTTC TAAACACCAA CCCCCACCCA	1860
TCCGGAACCT CCACCACTCC AACTTCAGCT TATCAGGTGC CCAGATAGAT GACAACAATC	1920
CAAGGCGTAC AGGCCACCGC ATTGTGGGCG CCCCTGGTGG CCGCTCCAAC ATCACCAGCC	1980
TCGGTTGACC TCAGATGAGC CAGATATGCA AGAGTGAAGG ATTATGGGAA AACGTCCATT	2040
CCTTTTCCGT GTTTTTGAAG CCCACAGTTT TAGTTGGTAC TGACGGAGGG GAGATTGAGC	2100
GATGCTCTTT CCTTCTCTGT TTAGGAAGAA GTGGTACTAG TGTGGTGTGT TTGCCTGGAA	2160
GTCCCTCGCC CACAGTGTGT GTTCACACCG ACTCCACCTC AGAGCATGGT GCCGTCCGTT	2220

- 51 -

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ser His Gln Gly Lys Lys Ser Ile Pro His Ile Thr Ser Asp Arg
 1 5 10 15
 Leu Leu Ile Arg Gly Gly Arg Ile Ile Asn Asp Asp Gln Ser Phe Tyr
 20 25 30
 Ala Asp Val Tyr Leu Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
 35 40 45
 Leu Ile Val Pro Gly Gly Val Lys Thr Ile Glu Ala Asn Gly Arg Met
 50 55 60
 Val Ile Pro Gly Gly Ile Asp Val Asn Thr Tyr Leu Gln Lys Pro Ser
 65 70 75 80
 Gln Gly Met Thr Ser Ala Asp Asp Phe Phe Gln Gly Thr Lys Ala Ala
 85 90 95
 Leu Ala Gly Gly Thr Thr Met Ile Ile Asp His Val Val Pro Glu Pro
 100 105 110
 Gly Ser Ser Leu Leu Thr Ser Phe Glu Lys Trp His Glu Ala Ala Asp
 115 120 125
 Thr Lys Ser Cys Cys Asp Tyr Ser Leu His Val Asp Ile Thr Ser Trp
 130 135 140
 Tyr Asp Gly Val Arg Glu Glu Leu Glu Val Leu Val Gln Asp Lys Gly
 145 150 155 160
 Val Asn Ser Phe Gln Val Tyr Met Ala Tyr Lys Asp Leu Tyr Gln Met
 165 170 175

- 52 -

Ser Asp Ser Gln Leu Tyr Glu Ala Phe Thr Phe Leu Lys Gly Leu Gly
180 185 190

Ala Val Ile Leu Val His Ala Glu Asn Gly Asp Leu Ile Ala Gln Glu
195 200 205

Gln Lys Arg Ile Leu Glu Met Gly Ile Thr Gly Pro Glu Gly His Ala
210 215 220

Leu Ser Arg Pro Glu Glu Leu Glu Ala Glu Ala Val Phe Arg Ala Ile
225 230 235 240

Ala Ile Ala Gly Arg Ile Asn Cys Pro Val Tyr Ile Thr Lys Val Met
245 250 255

Ser Lys Ser Ala Ala Asp Ile Ile Ala Leu Ala Arg Lys Lys Gly Pro
260 265 270

Leu Val Phe Gly Glu Pro Ile Ala Ala Ser Leu Gly Thr Asp Gly Thr
275 280 285

His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Ala Phe Val Thr Ser
290 295 300

Pro Pro Leu Ser Pro Asp Pro Thr Thr Pro Asp Tyr Leu Thr Ser Leu
305 310 315 320

Leu Ala Cys Gly Asp Leu Gln Val Thr Gly Ser Gly His Cys Pro Tyr
325 330 335

Ser Ile Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Thr Leu Ile Pro
340 345 350

Glu Gly Val Asn Gly Ile Glu Glu Arg Met Thr Val Val Trp Asp Lys
355 360 365

Ala Val Ala Thr Gly Lys Met Asp Glu Asn Gln Phe Val Ala Val Thr
370 375 380

Ser Thr Asn Ala Ala Lys Ile Phe Asn Leu Tyr Pro Arg Lys Gly Arg
385 390 395 400

Ile Ala Val Gly Ser Asp Ala Asp Val Val Ile Trp Asp Pro Asp Lys
405 410 415

Met Lys Thr Ile Thr Ala Lys Ser His Lys Ser Thr Val Glu Tyr Asn
420 425 430

Ile Phe Glu Gly Met Glu Cys His Gly Ser Pro Leu Val Val Ile Ser
435 440 445

Gln Gly Lys Ile Val Phe Glu Asp Gly Asn Ile Ser Val Ser Lys Gly
450 455 460

Met Gly Arg Phe Ile Pro Arg Lys Pro Phe Pro Glu His Leu Tyr Gln
465 470 475 480

Arg Val Arg Ile Arg Ser Lys Val Phe Gly Leu His Ser Val Ser Arg
485 490 495

Gly Met Tyr Asp Gly Pro Val Tyr Glu Val Pro Ala Thr Pro Lys His
500 505 510

Ala Ala Pro Ala Pro Ser Ala Glu Ser Ser Pro Ser Lys His Gln Pro
515 520 525

Pro Pro Ile Arg Asn Leu His Gln Ser Asn Phe Ser Leu Ser Gly Ala
530 535 540

Gln Ile Asp Asp Asn Asn Pro Arg Arg Thr Gly His Arg Ile Val Ala
545 550 555 560

Pro Pro Gly Gly Arg Ser Asn Ile Thr Ser Leu Gly
565 570

- 53 -

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1920 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

15 GCTGACTAAT ATGCTTAAAT TCAGCGGGTC GGCACGTCTG GTGGGTACGT CCACGCCCCG 60
GCAGCCCCCTA CCGAGGACAC TCAGCCCCGC CTTGTATCAG GATGTGCTTC CAAGGCAAGA 120
AGAGCAATCC CCGGATAACG AGCGACCGCC TTCTCATCAA AGGTGGGAAG ATTGTGAACG 180
ATGACCAGTC CTTTCATGCT GATCTGTATG TGGAAAGACGG TCTGATTAAA CAAATTGGAG 240
AAAATCTCAT CCTCCCTGGG GGCATCAAA CCATCGATGC TCATGCCCTG ATGGTGTCTG 300
CTGGGGGAGT TGACGTTTAC ACCCGGCTTC AGATGCTGTG GATGGGCATG ACCCCAGCTG 360
ATGATTTCG TCAGGSCACC AAGCGGGGTC TAGCAGGCGG GACCACCATG ATATTGGACC 420
ATGTGTTTCC TGACGCTGCT GTGAGCTTGC TGGCAGCCTA TGAGCAGTGG CCGGACGGAG 480
CAGACAGGCG GCGCTGCTGT GACTACTTCT TACATGTGGA CATTCCTCGC TGGCAGGAGA 540
GCACCAAGA AGAGCTGGAG GCGCTATCA GGGACAAAGG TGTGAATCC TTCCTGCTCT 600
TCATGGCATA CAAGGACAGG TGCCATGTGA CTGACGGTCA GATATATGAA ATCTTCAGCC 660
TCATCCGGA CCGTGGAGCT GTGGCCAGG TGCACGCAGA AAATGGGGAC ATCGTGGAGG 720
AGGAACAGAA GCGCTGCTG GAGCAAGGCA TCACTGCTCC TGAGGGCCAT GTGCTCAGCC 780
ACCCAGAAGA GGTAGAGGCC GAGCTGCTGT ACAGAGCAGT CACCATTGCC AAGCAGGCCA 840
ACTGCCCACT ATACGTCACC AAGCTGATGA GCAAGGGTGC AGCTGACATG GTTGCCCAAG 900
CCAAGCGCAG GGGGGTGGTC GTTTTGGGG AACCTATCAC TGCCAGCCTG GGCCTGATG 960
GCTCACACTA CTGGAGCAAG AATGGGCCA AGGCTGCAGC CTTTGTCACT TCACCCCTA 1020
TCACCCGGA CCGTACTACT GCAGACCACC TCACCTCTCT GCTGTCCAGT GGGACCTCC 1080
AGGTGACAGG CAGTGCCAC TGCACTTCA CTACTGCCCA GAAGGCTGTT GGCAAGACA 1140
ACTTCACACT GATGCCCGAG GTAGTCAAGG GTATAGAAGA CCGCATGCT GTGGTCTGGG 1200
AGAAATGTGT GGTTCAGGG AAAATGGAG AGAATCAGTT CGTGCGCTG ACCAGCACAA 1260
ATGCTGCCAA AATCTTCAT TTTACCCCA GGAAGGGCGG TGTGGCCGTG GGTCTGATG 1320
CTGACCTGCT CATCTGAAC CCGAGGGCCA CGAAATCAT CTCTGCCAAG AGCCATAACC 1380
TGAAATGAGA GTACAACATC TTTGAAGGAG TGGAGTGGG AGGAGTGGCC ACGGTGCTCA 1440
TAAGTCAGGG CAGAGTGTG CTGAGGAGG GAAACCTGCT TGTCACTCCA GGGGCTGGCC 1500
GCTTCATTCC CCGGAAGACG TTCCCGGACT TTGTCTATAA GAGGATAAAG GCTGCAACA 1560
GGCTAGCAGA GATCCACGGT GTGCTCGAG GCCTGTACGA CGGGCTGTG CATGAAGTGA 1620
TGTTACCTGC CAAGCCAGGA AGTGGCACAC AGGCGCGTGC ATCTGTTC AAGCAAGATCT 1680
CAGTGCCACC CGTGCGCAAC CTGCACCACT CCGGGTTCAG CTTATCTGCG TCTCAGGCTG 1740
ACCATCATAT TGGCAGACGT ACGGCTCAGA AGATCATGGC ACCCCCGGGA GGAGGCTCCA 1800
ACATCAGTC TCTTCTCTAG ACTTGGGGTC TTGGCAAGCT GGTGCTGTCC CCACTGGCAG 1860
GGTGTGGGGA GCACTCAGCT CAGTTAGCTC CTTCTTTGT AGATTGTTAT TGTGAAAGGC 1920

- 54 -

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

15

Met	Ser	Phe	Gln	Gly	Lys	Lys	Ser	Ile	Pro	Arg	Ile	Thr	Ser	Asp	Arg	1	5	10	15
Leu	Leu	Ile	Lys	Gly	Gly	Lys	Ile	Val	Asn	Asp	Asp	Gln	Ser	Phe	His	20	25	30	
Ala	Asp	Leu	Tyr	Val	Glu	Asp	Gly	Leu	Ile	Lys	Gln	Ile	Gly	Glu	Asn	35	40	45	
Leu	Ile	Val	Pro	Gly	Gly	Ile	Lys	Thr	Ile	Asp	Ala	His	Gly	Leu	Met	50	55	60	
Val	Leu	Pro	Gly	Gly	Val	Asp	Val	His	Thr	Arg	Leu	Gln	Met	Pro	Val	65	70	75	80
Met	Gly	Met	Thr	Pro	Ala	Asp	Asp	Phe	Cys	Gln	Gly	Thr	Lys	Ala	Ala	85	90	95	
Leu	Ala	Gly	Gly	Thr	Thr	Met	Ile	Leu	Asp	His	Val	Phe	Pro	Asp	Ala	100	105	110	
Gly	Val	Ser	Leu	Leu	Ala	Ala	Tyr	Glu	Gln	Trp	Arg	Asp	Gly	Ala	Asp	115	120	125	
Ser	Ala	Ala	Cys	Cys	Asp	Tyr	Ser	Leu	His	Val	Asp	Ile	Pro	Arg	Trp	130	135	140	
His	Glu	Ser	Thr	Lys	Glu	Glu	Leu	Glu	Ala	Leu	Val	Arg	Asp	Lys	Gly	145	150	155	160
Val	Asn	Ser	Phe	Leu	Val	Phe	Met	Ala	Tyr	Lys	Asp	Arg	Cys	Gln	Cys	165	170	175	
Thr	Asp	Gly	Gln	Ile	Tyr	Glu	Ile	Phe	Ser	Leu	Ile	Arg	Asp	Leu	Gly	180	185	190	
Ala	Val	Ala	Gln	Val	His	Ala	Glu	Asn	Gly	Asp	Ile	Val	Glu	Glu	Glu	195	200	205	

- 55 -

Thr	Lys	Val	Ile	Ser	Ala	Lys	Ser	His	Asn	Leu	Asn	Val	Glu	Tyr	Asn	
			420					425					430			
Ile	Phe	Glu	Gly	Val	Glu	Cys	Arg	Gly	Val	Pro	Thr	Val	Val	Ile	Ser	
		435					440					445				
Gln	Gly	Arg	Val	Val	Leu	Glu	Asp	Gly	Asn	Leu	Leu	Val	Thr	Pro	Gly	
	450					455					460					
Ala	Gly	Arg	Phe	Ile	Pro	Arg	Lys	Thr	Phe	Pro	Asp	Phe	Val	Tyr	Lys	
465					470					475					480	
Arg	Ile	Lys	Ala	Arg	Asn	Arg	Leu	Ala	Glu	Ile	His	Gly	Val	Pro	Arg	
			485						490					495		
Gly	Leu	Tyr	Asp	Gly	Pro	Val	His	Glu	Val	Met	Leu	Pro	Ala	Lys	Pro	
			500					505					510			
Gly	Ser	Gly	Thr	Gln	Ala	Arg	Ala	Ser	Cys	Ser	Gly	Lys	Ile	Ser	Val	
		515					520					525				
Pro	Pro	Val	Arg	Asn	Leu	His	Gln	Ser	Gly	Phe	Ser	Leu	Ser	Gly	Ser	
	530					535					540					
Gln	Ala	Asp	Asp	His	Ile	Ala	Arg	Arg	Thr	Ala	Gln	Lys	Ile	Met	Ala	
545					550					555					560	
Pro	Pro	Gly	Gly	Arg	Ser	Asn	Ile	Thr	Ser	Leu	Ser					
			565					570								

- 56 -

INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1690 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gln	Lys	Arg	Leu	Leu	Glu	Gln	Gly	Ile	Thr	Gly	Pro	Glu	Gly	His	Val	210	215	220
Leu	Ser	His	Pro	Glu	Glu	Val	Glu	Ala	Glu	Ala	Val	Tyr	Arg	Ala	Val	225	230	235 240
Thr	Ile	Ala	Lys	Gln	Ala	Asn	Cys	Pro	Leu	Tyr	Val	Thr	Lys	Val	Met	245	250	255
Ser	Lys	Gly	Ala	Ala	Asp	Met	Val	Ala	Gln	Ala	Lys	Arg	Arg	Gly	Val	260	265	270
Val	Val	Phe	Gly	Glu	Pro	Ile	Thr	Ala	Ser	Leu	Gly	Thr	Asp	Gly	Ser	275	280	285
His	Tyr	Trp	Ser	Lys	Asn	Trp	Ala	Lys	Ala	Ala	Ala	Phe	Val	Thr	Ser	290	295	300
Pro	Pro	Ile	Asn	Pro	Asp	Pro	Thr	Thr	Ala	Asp	His	Leu	Thr	Ser	Leu	305	310	315 320
Leu	Ser	Ser	Gly	Asp	Leu	Gln	Val	Thr	Gly	Ser	Ala	His	Cys	Thr	Phe	325	330	335
Thr	Thr	Ala	Gln	Lys	Ala	Val	Gly	Lys	Asp	Asn	Phe	Thr	Leu	Ile	Pro	340	345	350
Glu	Val	Val	Asn	Gly	Ile	Glu	Glu	Arg	Met	Ser	Val	Val	Trp	Glu	Lys	355	360	365
Cys	Val	Ala	Ser	Gly	Lys	Met	Asp	Glu	Asn	Glu	Phe	Val	Ala	Val	Thr	370	375	380
Ser	Thr	Asn	Ala	Ala	Lys	Ile	Phe	Asn	Phe	Tyr	Pro	Arg	Lys	Gly	Arg	385	390	395 400
Val	Ala	Val	Gly	Ser	Asp	Ala	Asp	Leu	Val	Ile	Trp	Asn	Pro	Arg	Ala	405	410	415

- 57 -

GCGGTTGCTG GAGCTCGGCA TCACTGGCCC CGAGGGCCAC GTGCTCGCC ACCCCGAGGA 720
GGTGGAGGCT GAGGCGGTGT ACCGAGCTGT CACCATCGCC AAGCAGGCAA ACTGCCCCTG 780
GTACGTCACC AAGGTGATGA GCAAGGGGGC GGCCGACGCC ATCGCTCAGG CCAAGCGCAG 840
AGGGGTGGTC GTGTTTGGGG AGCCCATCAC CGCCAGCCTG GGCACCGACG GTTCACACTA 900
CTGGAGCAAG AACTGGGCCA AGGCTGCAGC CTTCGTACA TCACCCCTG TCAACCCAGA 960
CCCCACCACG GCAGACCACC TCACCTGCTT GCTGTCCAGC GGGGACCTCC AGGTGACAGG 1020
CAGCGCCAC TGCACCTCA CCACTGCCCA GAAGGCTGTG GGCAAGGACA ACTTCGCGCT 1080
GATCCCCGAG GGCACCAACG GCATTGAGGA GCGCATGTG ATGGTCTGGG AGAAATGTGT 1140
GGCCTCTGGG AAGATGGACG AGAATGAGTT CGTCGCGGTG ACCAGTACAA ATGCTGCCAA 1200
AATCTTCAAT TTTTACCCAA GGAAGGGGCG AGTGGCTGTG GGCTCTGACG CTGACCTGGT 1260
CATATGGAAC CCCAAGGCCA CCAAGATCAT CTCTGCCAAG ACCACAATC TGAACGTGGA 1320
GTACAACATC TTCGAGGGAG TGGAGTCCCG GGGAGCGCCT GCCGTGGTCA TAAGTCAGGG 1380
CCGAGTGGCG CTGGAGGACG GGAAGATGTT TGTACCCCG GGGGCGGGCC GCTTCGTCC 1440
TCGGAAAACA TTCCCGGACT TTGTCTACAA GAGGATCAA GCTCGCAACA GGCTGGCGGA 1500
GATCCACGGT GTGCCCCGTG GGCTGTATGA CGGGCCCGTC CACGAGGTGA TGGTGCTGC 1560
CAAGCCAGGG AGTGGCGCTC CGGCCCGCGC GTCCTGCCCA GGCAAGATCT CCGTGCCCTCC 1620
TGTGCGCAAC CTACATCACT CGGGGTTTCA CTTATCTGGG TCTCAGGCTG ATGACCACAT 1680
CGCCCGACGC 1690

- 58 -

INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 572 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Ser Phe Gln Gly Lys Lys Ser Ile Pro Arg Ile Thr Ser Asp Arg
1 5 10 15
Leu Leu Ile Arg Gly Gly Arg Ile Val Asn Asp Asp Gln Ser Phe Tyr
20 25 30
Ala Asp Val His Val Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
35 40 45
Leu Ile Val Pro Gly Gly Ile His Thr Ile Asp Ala His Gly Leu Met
50 55 60
Val Leu Pro Gly Gly Val Asp Val His Thr Arg Leu Gln Met Pro Val
65 70 75 80
Leu Gly Met Thr Pro Ala Asp Asp Phe Cys Gln Gly Thr Lys Ala Ala
85 90 95
Leu Ala Gly Gly Thr Thr Met Ile Leu Asp His Val Phe Pro Asp Thr
100 105 110
Gly Val Ser Leu Leu Ala Ala Tyr Glu Gln Trp Arg Glu Arg Ala Asp
115 120 125
Ser Ala Ala Cys Cys Asp Tyr Ser Leu His Val Asp Ile Thr Arg Trp
130 135 140
His Glu Ser Ile Lys Glu Glu Leu Glu Ala Leu Val Lys Glu Lys Gly
145 150 155 160
Val Asn Ser Phe Leu Val Phe Met Ala Tyr Lys Asp Arg Cys Gln Cys
165 170 175
Ser Asp Ser Gln Met Tyr Glu Ile Phe Ser Ile Ile Arg Asp Leu Gly
180 185 190
Ala Leu Ala Gln Val His Ala Glu Asn Gly Asp Ile Val Glu Glu Glu
195 200 205
Gln Lys Arg Leu Leu Glu Leu Gly Ile Thr Gly Pro Glu Gly His Val
210 215 220

- 59 -

Leu Ser His Pro Glu Glu Val Glu Ala Glu Ala Val Tyr Arg Ala Val
225 230 235 240

Thr Ile Ala Lys Gln Ala Asn Cys Pro Leu Tyr Val Thr Lys Val Met
245 250 255

Ser Lys Gly Ala Ala Asp Ala Ile Ala Gln Ala Lys Arg Arg Gly Val
260 265 270

Val Val Phe Gly Glu Pro Ile Thr Ala Ser Leu Gly Thr Asp Gly Ser
275 280 285

His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Phe Val Thr Ser
290 295 300

Pro Pro Val Asn Pro Asp Pro Thr Thr Ala Asp His Leu Thr Cys Leu
305 310 315 320

Leu Ser Ser Gly Asp Leu Gln Val Thr Gly Ser Ala His Cys Thr Phe
325 330 335

Thr Thr Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Ala Leu Ile Pro
340 345 350

Glu Gly Thr Asn Gly Ile Glu Glu Arg Met Ser Met Val Trp Glu Lys
355 360 365

Cys Val Ala Ser Gly Lys Met Asp Glu Asn Glu Phe Val Ala Val Thr
370 375 380

Ser Thr Asn Ala Ala Lys Ile Phe Asn Phe Tyr Pro Arg Lys Gly Arg
385 390 395 400

Val Ala Val Gly Ser Asp Ala Asp Leu Val Ile Trp Asn Pro Lys Ala
405 410 415

Thr Lys Ile Ile Ser Ala Lys Thr His Asn Leu Asn Val Glu Tyr Asn
420 425 430

Ile Phe Glu Gly Val Glu Cys Arg Gly Ala Pro Ala Val Val Ile Ser
435 440 445

Gln Gly Arg Val Ala Leu Glu Asp Gly Lys Met Phe Val Thr Pro Gly
450 455 460

Ala Gly Arg Phe Val Pro Arg Lys Thr Phe Pro Asp Phe Val Tyr Lys
465 470 475 480

Arg Ile Lys Ala Arg Asn Arg Leu Ala Glu Ile His Gly Val Pro Arg
485 490 495

Gly Leu Tyr Asp Gly Pro Val His Glu Val Met Val Pro Ala Lys Pro
500 505 510

Gly Ser Gly Ala Pro Ala Arg Ala Ser Cys Pro Gly Lys Ile Ser Val
515 520 525

Pro Pro Val Arg Asn Leu His Gln Ser Gly Phe Ser Leu Ser Gly Ser
530 535 540

Gln Ala Asp Asp His Ile Ala Arg Arg Thr Ala Gln Lys Ile Met Ala
545 550 555 560

Pro Pro Gly Gly Arg Ser Asn Ile Thr Ser Leu Ser
565 570